2/11/86

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

004937

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: RH-3866 2E Fungicide (EPA #707-EUP-RNU)
RH-3866 40W Fungicide (EPA #707-EUP-RNL)
EPA Pesticide Petition 4G3149
Application for Experimental Use Permit (EUP) and Temporary Tolerance; Sythane 40W Fungicide (EPA Registration No. 707-ROG); Application for Use on Perennial Grasses Grown for Seed; Other Names Include RH-53,866 and Myclobutanil

Caswell No. 723K

FROM: Jane E. Harris, Ph.D., Head, Section VI JEH 2/11/86
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Henry M. Jacoby, PM 21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Theodore M. Farber, Ph.D., Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Applicant: Rohm & Haas Co.
Philadelphia, Pennsylvania

Submission Purpose:

Registrant has requested a temporary tolerance for RH-3866 Fungicide on apples and grapes at 0.5 part per million (ppm) for the fresh market and for turf use with restrictions on grazing of livestock. Original request for use on wheat for feed or bedding purposes and for the RH-3866 2E formulation have all been withdrawn, accompanied by the deletion of the original request for temporary tolerances on meat, milk, and eggs. As a result of these changes, the proposed annual use of RH-3866 is 2624 pounds

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active ingredient (lbs ai) of which 54 percent, 36 percent. and 21 percent for use on apples, grapes, and perennial grasses grown for seed, respectively, for approximately 1620 acres. This application involves both the RH-3866 technical and the RH-3866 40W formulation. RH-3866 40W is intended to control powdery mildew and scab on apples, mildew, and blackrot on grapes and certain foliar diseases of perennial turf grasses grown for seed production. The effective use rate ranges from 0.05 to 0.25 lb ai per acre for single and multiple applications with a total accumulation of active ingredient not to exceed 2 lbs per acre in a single season. Conventional ground spray equipment is recommended with a postharvest interval for fresh market apples and grapes of 14 days and for turf grown for seed of at least 21 days.

Recommendation:

The Toxicology Branch considers the data adequate to support temporary tolerances for RH-3866 40W Fungicide for apples and grapes at 0.5 ppm (707-EUP-RNL) and for RH-3866 40W on perennial turf grown for seed (707-ROG) with restrictions on grazing of livestock. Support for this EUP for RH-3866 is contingent on the concurrence by the Exposure Assessment Branch of the risk analysis (presented in Appendix 1) for mixer/loaders with respect to potential for testicular lesions; testicular atrophy was observed in tne second generation of the reproduction study in rats at 1000 ppm and at 12 and 17 months of the 2-year feeding study in rats at 800 ppm.

All the lowest effect levels observed in the subchronic rat, subchronic and one-year dog, and 2-generation reproduction study in rats were similarly based on increased liver weights and hepatic hypertrophy supporting the lowest NOEL in the dog of 100 ppm or 2.5 mg/kg bwt/day. A provisional acceptable daily intake (PADI) is calculated to be 0.0025 mg/kg bwt/day (2.5 mg/kg bwt/day/1000 safety factor), using up 15.1 percent of the TMRC of 0.0227 mg/day (1.5 kg diet) for temporary tolerances on apples and grapes for the fresh market.

In general, acute toxicity studies indicate category III in toxicity for the technical and 40 WP formulation with the exception of severe eye irritation (category I) for RH-3866 technical, and only mild to no dermal irritation (category IV) for technical and formulation. The label requiring the use of splash goggles provides sufficient protection for the eye-irritating effects of RH-3866. A dermal sensitization study in the guinea pig was negative for the 2EC formulation containing 24 percent ai RH-3866.

In an attempt to protect the farmworker against potential testicular lesions observed at the HDT in rats in the second generation of the reproduction study after exposure *in utero* and during lactation at 1000 ppm or 80 mg/kg bwt/day, and by 12 months 2

in the 2-year rat feeding study at 800 ppm or 40 mg/kg bwt/day, the amended label now requires new protective clothing (long trousers, long-sleeved shirts), impervious gloves, and splash goggles. A conservative analysis of risk to the mixer/loader, as presented in Appendix 1 below, provides approximate margins of safety of 1887 and 326 to the worker with or without protective clothing, respectively. The margin of safety is calculated from anticipated acute daily exposures to the mixer/loader and a NOEL of 200 ppm (10 mg/kg bwt/day) based on testicular effects observed after repeated daily exposure to dietary levels of 800 to 1000 ppm (40 to 80 mg/kg bwt) at one year in a chronic rat feeding study and in the second generation of a rat reproduction study.

In addition to testicular effects discussed above, a rat teratology study with RH-3866 technical showed embryotoxicity at 94 mg/kg bwt/day (increased resorptions and decreased viability indices) and fetotoxicity at 313 mg/kg bwt/day (increased incidences of 14th rudimentary and 7th cervical ribs), supporting an overall NOEL for developmental toxicity of 31 mg/kg bwt/day. Mutagenicity testing showed no mutagenic activity in an Ames test, Chinese Hamster ovary/HGPRT point mutation assay or an in vivo cytogenicity assay in mice of RH-3866 technical. To satisfy requirements for permanent registration of mutagenicity testing, the registrant should perform a DNA damage/repair assay, and a dominant lethal assay to assess potential acute toxicity to the germinal cells of male rats in view of the observed testicular lesions in the 2-generation reproduction and chronic rat feeding studies. Finally, in satisfying requirements for permanent registration, attention should be given to any testicular effects in the 21-day dermal exposure to RH-3866. Both the dominant lethal study and 21-day dermal exposure study should assist us in interpreting the potential for testicular lesions in farmworkers exposed to RH-3866.

Related Actions:

Reviews of the 2-generation reproduction study and interim report of the 2-year chronic feeding study in rats and preliminary findings in the one-year feeding study in dogs:

Sythane or Myclobutanil/RH-53,866 or RH-3866, EPA I.D. Nos. 4G-3149/707-EUP-RNL/707-ROG; Accession Nos. 073522, 073805, 073806, 073807.

Chemicals:

RH-3866 or RH-53,866 Technical ~81 to 84.5% ai: α-butyl-α-4-chlorophenyl-1-H-1,2,4-triazole-1-propanenitrile

Formulation: RH-3866 40W Fungicide (40% ai)

RH-3866 2E Fungicide (24% ai) withdrawn

Toxicity Data

RH-3866 Technical (91% ai)
Tox. Category/Core Classification

Acute Oral LD_{50} (rat): Male 1.6 g/kg; III/minimum
Female 2.3 g/kg

Acute Oral LD_{50} (mouse): Male > 4.42 g/kg; III/Supplementary

Acute Dermal LD_{50} (rabbits): > 5 g/kg; III/Minimum

Primary Dermal Irritation (rabbits): Nonirritating; IV/Minimum

Primary Eye Irritation (rabbits): Severe Irritant I/Minimum

RH-3866 Technical (81% ai)

Subchronic 13-week Oral Rat (Sprague-Dawley):
NOEL: 1000 ppm (50 mg/kg bwt/day)
LEL: 3000 ppm (150 mg/kg bwt/day)
(increased absolute and relative liver weights, increased relative kidney weights, hypertrophy and necrosis in liver and pigmentation in convoluted kidney tubules.)

Levels Tested: 100, 300, 3000, 10,000, 30,000 ppm.

Core Minimum

Subchronic/13-week oral Dog (Beagle):

	Male	Female
NOEL:	10 ppm (2.5 mg/kg)	200 ppm (5 mg/kg)
LEL:	200 ppm (5 mg/kg)	800 ppm (20 mg/kg)

(hepatic hypertrophy in both sexes, increased alkaline phosphatase at 800 ppm in females and males)

Levels Tested: 10, 200, 800, 1600 ppm

Core Minimum

	RH-3866 Technical (84-85% ai)
One-year Oral Dog (Beagle) (Preliminary report)	NOEL (liver) 100 ppm (2.5 mg/kg bwt/day) LEL (liver) 400 ppm (10 mg/kg bwt/day) (hepatic hypertrophy in both sexes and increased liver weights in females) Levels tested: 10, 100, 400, 1600 ppm
Two-generation Reproduction Study in Rat (Sprague-Dawley)	Systemic NOEL: 50 ppm (4 mg/kg bwt/day) LEL: 200 ppm (16 mg/kg bwt/day) (increased absolute and relative liver weights and hepatic hypertrophy in males) Reproductive NOEL: 200 ppm (16 mg/kg bwt/day) LEL: 1000 ppm (80 mg/kg bwt/day) (testicular, epididymal and prostatic atropy in P_2 males; increased number of stillborns, decreased weight gain in pups during lactation in F_1 and F_2) Levels tested: 50, 200 and 1000 ppm
	Core Guideline
Rat Teratology Study (Sprague-Dawley)	Teratogenic NOEL: > 469 mg/kg bwt Maternal NOEL: 313 mg/kg bwt LEL: 469 mg/kg bwt (decreased body weight gain, clinical signs) Embryotoxic NOEL: 31 mg/kg bwt LEL: 94 mg/kg bwt (increased resportions and decreased viability index) Fetotoxic NOEL: 94 mg/kg bwt LEL: 313 mg/kg bwt (increased 7th cervical and 14 rudimentary ribs) Levels Tested: 31, 94, 313, 469 mg/kg bwt/day
	Core Minimum
Mutagenicity Assay Ames Test	(RH-3866 Technical) (90% ai); Negative/Acceptable
Point Mutation CHO/HGPRT	(81% ai); Negative/Acceptable

In vivo Cytogenetic Assay (81% ai) Negative/Acceptable

Formulation:	RH-3866 2EC (24% ai) (Withdrawn)
	Toxicity Category/Core Classification
Acute Oral LD_{50} (rat)	Males 1.8 g/kg; III/Minimum Females 1.3 g/kg
Acute Dermal LD_{50} (rabbit)	$\geq$ 5 g/kg; III/Minimum
Primary Dermal Irritation (rabbit)	Severe Irritation; II/Minimum
Primary Eye Irritation (rabbit)	Severe Irritation; I/Minimum
Acute Inhalation LC_{50} (rat)	> 4 mg/L; III/Minimum
Dermal Sensitization (guinea pig)	No Hypersensitivity/Guideline
Formulation:	RH-3866 40 W - (wettable powder) (40% ai) Toxicity Category/Core Classification
Acute Oral LD_{50} (rat)	Male 1.9 g/kg bwt; III/Minimum Female 2.1 g/kg bwt
Acute Dermal LD_{50} (rabbit)	> 5 g/kg bwt; III/Minimum
Primary Dermal Irritation (rabbit)	Mild Irritant; IV/Minimum
Primary Eye Irritation (rabbit)	Moderate Irritant; III/Supplementary
Acute Inhalation LC_{50} (rat)	> 5 mg/L; III/Minimum

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____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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EPA: 68-02-4225
DYNAMAC No. 33C-1,2
January 20, 1986

DATA EVALUATION RECORD

RH-3866

Subchronic Oral Toxicity Study in Dogs

STUDY IDENTIFICATION: O'Hara, G., McLaughlin, J., and DiDonato, L. RH-3866: A three-month dietary study in dogs. (Unpublished study No. 83R-204 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated August 7, 1984.) Accession No. 072899-072900.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 1-26-86

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1. CHEMICAL: RH-3866; RH-53866; α-butyl-α-(4-chlorophenyl)-1H-1,2,-4-triazole-1-propanenitrile.

2. TEST MATERIAL: RH-3866 technical, sample No. TD 83-076, lot No. LSPL0016/E, was described as a brown solid containing 81.1 percent active ingredient.

3. STUDY/ACTION TYPE: Subchronic oral toxicity study in dogs.

4. STUDY IDENTIFICATION: O'Hara, G., McLaughlin, J., and DiDonato, L. RH-3866: A three-month dietary study in dogs. (Unpublished study No. 83R-204 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated August 7, 1984.) Accession No. 072899-072900.

5. REVIEWED BY:

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Jane E. Harris, Ph.D.
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Signature: Jane E. Harris
Date: 1/22/86

7. CONCLUSIONS:

A. The NOEL for RH-3866 based on this 3-month feeding study in dogs is 10 ppm. The LOEL for male dogs is 200 ppm based on liver centrilobular or midzonal hepatocellular hypertropy. The LOEL for female dogs for the same endpoint is 800 ppm. Higher doses produced hematological alterations associated with a decrease in red blood cell count (RBC), hemoglobin (Hb), and hematocrit (HCT) and increase in mean corpuscular hemoglobin (MCH), MC volume (MCV), MCH concentration (MCHC), and platelet counts. The alkaline phosphatase level was increased at 800 ppm for both sexes, which correlated with the above liver alteration, and there was an increase in absolute liver weight (800-ppm group) and ratios (1600-ppm group). Decreased body weight (weeks 2 and 3) and food consumption were also observed at 1600 ppm.

B. Core Classification: Core Minimum.

Items 8 and 10--see footnote 1.

9. BACKGROUND: Previous acute toxicity studies showed that RH-3866 was slightly toxic to rats (LD_{50} <1.3 g/kg) and mice (LD_{50} = 3.23 g/kg) following ingestion of a single oral dose, was practically nontoxic to rabbits following a single dermal application, was practically nonirritating to rabbit skin, and was only slightly irritating to the eyes of rabbits. A subchronic feeding study in rats dosed with RH-3866 technical showed compound-related signs of toxicity, primarily in the liver and kidney, at dietary concentrations of 1000 ppm and greater.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. The test material was RH-3866, lot No. LSPL0016/E, a technical material containing 81.1 of the active ingredient α-butyl-α-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile. Aliquots of the test material were warmed, and 50 ml acetone solutions were prepared, mixed with the dry food, and fed at 0, 10, 200, 800, and 1600 ppm. The test diet was analyzed for RH-3866 content at various intervals during the study. Samples were also taken and analyzed for stability and homogeneity in the diet.

2. The dietary ration for this study was Purina Dog Chow Meal No. 5006. Dogs received a 300-g offering over 2 hours, after which the remaining food was removed. Water was supplied ad libitum.

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[1]Only items appropriate to this DER have been included.

3. The dogs used in this study (four/sex/dose group) were 5-month-old beagle dogs from Marshall Research Animals, North Rose, NY. They were housed individually in stainless steel cages. Environmental conditions were controlled for temperature, humidity, and light.

4. Daily observations were made for mortality, morbidity, and signs of toxicity. Physical examinations were made weekly for the first 4 weeks and every other week thereafter. Food consumption was measured daily and body weights were measured weekly. Hematology, consisting of 10 parameters, and clinical chemistry, consisting of 15 parameters, were evaluated twice prior to dosing and at 4 and 13 weeks. Urinalysis was not performed. Ophthalmoscopic examinations were made at pretest and termination.

5. Necropsies were performed at termination and approximately 37 tissues were taken for histologic evaluation. Histopathologic examination was limited to high-dose and control dogs of both sexes. Organ weights were measured on nine selected organs.

6. The results were examined for normality and homogeneity of variance, transformed as necessary, and assessed for presence or absence of an overall treatment effect by analysis of covariance. Group means were compared using a t-test. Organ weights were analyzed using Duncan's multiple range test.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. No measurable concentration of the test compound was found in the control diet when analyzed (sensitivity, 1.0 ppm). According to the authors, dose levels approximated expected dose levels within 10 percent. In fact, 12 of the 27 values presented in the detailed residue data varied by more than 10 percent of the nominal dose. The mixed diets were analyzed for homogeneity and the results indicated that variability was about 10-15 percent.

B. No deaths occurred during the study, and it was reported that no compound-related signs of toxicity were noted. Incidental clinical signs included changes in the stool and/or emesis in all groups including the control. Single incidences of lacrimation, scant feces, and lacerations were observed and were judged not to be compound related. The above comments (except for survival) are based on authors' conclusions. No individual data or summary incidence of signs of toxicity were presented on which to judge the authors' statements. According to the authors, physical

examination revealed no unusual findings in the dogs regardless of the dose they received. However, no detailed data were provided. No compound-related effects on heart rate or body temperature were observed.

C. Mean body weights (Table 1 from the CBI, see Appendix C) were significantly lower ($p < 0.05$) than the controls in the high-dose males during the first 2 weeks and in high-dose females for the first 3 weeks of the study. Food consumption (Table 2 from the CBI, see Appendix C) was significantly reduced ($p < 0.05$) in the high-dose males throughout the first 7 weeks and in the high-dose females for the entire study (see Table 2 from the CBI, see Appendix C). The authors judged that the effect was one of palatability of the test substance. The body weight or food consumption of animals receiving 800 ppm were not affected. A summary table for body weight gain and food consumption is provided in Table 1 of the text.

D. Ophthalmoscopic examination revealed no ocular abnormalities that could be attributable to ingestion of RH-3866 over a 3-month period.

E. Table 2 presents selected hematology data. There were increases in mean platelet counts in males and females receiving 1600 ppm; these may have been related to dosing. RBC, HCT, and Hb were slightly but significantly decreased at 1 and 3 months in males receiving 1600 ppm but not in females.

F. Table 3 presents those clinical chemistry values found to be significantly different from the controls and where some dose relationship was evident. A dose-related increase in serum alkaline phosphatase was seen in both sexes at doses of 800 and 1600 ppm; this effect may correlate with histologic liver changes (see below). Gamma glutamyl transferase activity was slightly but significantly increased compared to control values at 1 month but not 3 months in males receiving 800 ppm and at both intervals in females receiving 1600 ppm; these increases were considered too small to indicate a toxicologic effect on the liver. Serum glutamic pyruvic transaminase (SGPT) levels were significantly decreased in high-dosed females; however, the values were within the pretest range and the effects were not dose-related and were considered unimportant since decreased levels do not correlate with any organ effects. There were random fluctuations in blood urea nitrogen (BUN), cholesterol, phosphorus, creatinine, and calcium, which were considered not of toxicologic importance but due to the small sample size (four/sex/group).

G. No consistent gross necropsy findings could be related to the administration of the test compound at any dosage level.

TABLE 1. Summary of Body Weight Gain and Food Consumption in Dogs Fed RH-3866 for 14 Weeks

Dietary Level (ppm)	Mean Weight Gain (kg) between Weeks			
	0-2	0-14	0-3	0-14
	Males		Females	
0	+0.09	+0.13	+0.28	+0.35
1600	-0.17	+0.19	-0.29	-0.09

	Selected Mean Food Consumption (g/week)			
	Weeks			
	1	4	7	14
Males				
0	2093.3	2100.0	2100.0	2100.0
1600	1659.8*	1815.5*	1984.5*	2100.0
Females				
0	1920.8	2076.3	2100.0	2100.0
1600	1446.3*	1638.5*	1780.3*	1823.0*

*Significantly different from the control value ($p<0.05$).

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TABLE 2. Selected Mean Hematologic Values at Selected Intervals for Male and Female Dogs Administered RH-3866 in the Diet for 3 Months

Parameter/ Dose (ppm)	Males at day			Females at day		
	-5	29	97	-5	29	97
RBC (10^6/mm^3)						
0	6.407	7.100	7.220	6.700	7.337	7.332
800	6.465	7.015	7.360	7.047	7.427	7.235
1600	5.945	6.522*	6.685*	6.505	7.182	6.762†
HCT (%)						
0	48.12	51.72	54.02	49.70	53.60	54.17
800	48.25	51.30	55.77	51.65	53.67	53.77
1600	44.42	48.50*	50.65*	49.02	52.30	51.52
Hb (g/dL)						
0	13.75	14.25	14.90	14.27	14.72	14.77
800	13.85	14.20	15.30	14.52	14.60	14.67
1600	12.75	13.57*	14.20*	13.95	14.92	14.37
Platelet (10^3/mm^3)						
0	314.0	294.3	303.8	351.8	343.8	350.3
800	306.5	302.3	321.8	365.8	357.3	390.3
1600	284.3	338.8†	349.8*†	306.0	368.8*†	409.5*†
MCH (µµg)						
0	21.47	20.35	20.62	21.30	20.10	20.22
800	21.42	20.27	20.82†	20.65	19.67	20.35†
1600	21.47	20.50	21.27*†	21.42	20.77	21.25†
MCV (m^3)						
0	75.0	73.8	74.5	74.0	73.0	74.0
10	76.5	75.3†	77.3*†	75.3	74.8*	76.0*†
200	74.8	73.5	74.5	75.5	74.3	75.5
800	74.8	73.8	75.8*†	73.5	72.3	74.5†
1600	75.0	73.3	76.0*†	75.3	74.3	76.0*†

*Significantly different from control value ($p < 0.05$).

†Significantly different from control value when sexes were combined and analyz... ($p < 0.05$).

TABLE 3. Selected Mean Clinical Chemistry Values at Selected Intervals for Male and Female Dogs Administered RH-3866 in the Diet for 3 Months

Parameter/ Dose (ppm)	Males at day			Females at day		
	-5	29	97	-5	29	97
Alkaline phosphatase (U/L)						
0	116.8	88.8	74.3	83.5	65.0	67.3
200	95.3	77.0	78.8	94.5	102.8	93.3
800	80.0	79.3	93.8*	77.3	107.5*	110.3
1600	87.0	91.8*	109.3*	99.0	160.0*	233.0*
Gamma glutamyl transferase (U/L)						
0	1.0	1.0	1.5	0.0	0.0	0.0
10	0.5	2.3	0.0*	0.7	1.5	0.0
200	0.5	1.3	0.5	0.5	1.0	0.5
800	0.0	3.5*†	1.0	0.5	1.8†	1.0
1600	1.0	2.0†	0.0*	0.0	3.5*†	1.5*

*Signicantly different from control value ($p < 0.05$), sexes analyzed separately.

†Signicantly different from control value ($p < 0.05$), sexes analyzed together.

H. The absolute and relative liver weights (Table 4) of male and female dogs receiving RH-3866 at 1600 ppm were significantly ($p < 0.05$) greater than controls. Also, the liver weights of the male dogs receiving 800 ppm were significantly ($p < 0.05$) larger than the controls. Other significant ($p < 0.05$) organ weight changes included increased absolute brain weight in the 10-ppm males, increased absolute pituitary weight in 200-ppm females, and increased absolute and relative spleen weight in the 200-ppm females. Though not significantly different from controls, the gonad weights in the 800- and 1600-ppm females were increased. Except for the liver weights none of the above were considered meaningful because of a lack of a dose/effect relationship. Also, the females were in estrus, accounting for the gonadal changes.

I. The incidence of minimal to mild centrilobular or midzonal hepatocytic hypertrophy indicated that these lesions were associated with the administration of the test compound (Table 5). The effect on the liver was apparent in the males receiving 200 ppm and above and in the females receiving 800 ppm and above. The incidence of chronic interstitial nephritis was increased in males receiving 200 ppm and above.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Administration of RH-3866 in the diets of dogs for 3 months produced several compound-related, but not adverse, toxicologic effects. Decreased body weight and food consumption (1600-ppm males and females) were considered to be a result of low palatability of treated feed at this concentration. Changes in hematology and blood chemistry were not considered to be toxicologically significant. The authors concluded that the effects on the liver (increased alkaline phosphatase activity, increased absolute and relative liver weights, and centrilobular and midzonal hepatocellular hypertrophy) were adaptive changes in response to an increased hepatic workload caused by the test material.

The NOEL was determined to be 10 ppm (0.34 mg/kg/day) for male dogs and 200 ppm (7.88 mg/kg/day) for female dogs based on histologic changes in the liver. The NOAEL (no observed adverse effect level) was determined to be 1600 ppm for males and females.

B. A signed, but not dated, quality assurance statement was presented.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. The study authors concluded that the actual dose levels approximated the nominal dose levels within 10 percent. Review of

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TABLE 4. Mean Liver Weights and Liver-to-Body Weight Ratios for Male and Female Dogs Administered RH-3866 in the Diet for 3 Months[a]

Dose Group (ppm)	Liver Weight (g)	Liver Weight/ Body Weight
	MALES	
0	255.52	0.032
10	266.99	0.030
200	293.97	0.035
800	334.73*	0.039
1600	358.92*	0.045*
	FEMALES	
0	250.07	0.036
10	234.05	0.032
200	264.55	0.035
800	297.06	0.040
1600	322.63*	0.047*

* Significantly different from control ($p < 0.05$) using ANOVA followed by Duncan's test for multiple comparisons (as calculated by our reviewers).

[a]No significant differences were indicated in the report.

TABLE 5. Incidence (degree of severity[a]) of Centrilobular and Midzonal Hepatocytic Hypertrophy or Chronic Interstitial Nephritis in Male and Female Dogs Administered RH-3866 in the Diet for 3 Months

Dose (ppm)	Centrilobular or midzonal hepatocytic hypertrophy		Chronic interstitial nephritis	
	Total	Distribution	Total	Distribution
		Males		
0	0	0	1	1 (+)
10	0	0	1	1 (2+)
200	3	3 (+)	3	2 (+), 1 (3+)
800	4	1 (+), 3 (2+)	3	2 (+), 1 (2+)
1600	4	4 (2+)	3	2 (+), 1 (2+)
		Females		
0	0	0	2	2 (+)
10	0	0	2	2 (+)
200	0	0	2	2 (+)
800	4	4 (+)	2	2 (+)
1600	4	2 (+), 2 (2+)	2	2 (+)

[a] (+) minimal; (2+) mild; (3+) moderate; (4+) severe.

the data on retained feed indicates that the dose varied from the intended dose by a factor greater than 10 percent. In 15 out of 28 cases, or 54% of the time, the dose varied from the nominal dose. Nevertheless, the variation was related primarily to the highest dose and was not so great as to compromise the study.

B. The administration of the test material had no effect on mortality, signs of toxicity, body temperature, heart rate, and the results of physical examination or ophthalmoscopic examination.

C. The lower RBC, HCT, and Hb values together with the higher MCH, MCV, and MCHC are all suggestive of mild red cell destruction or mild anemia. Except for MCV, the effect was limited to the 1600-ppm dose group and was more apparent in the males than the females. The effect was limited in severity.

This effect was noted in the rat study and should have led to reticulocyte assays being incorporated in the design of this study. There was evidence of hemosiderosis in the spleen of two high-dose male dogs, as would be expected in red cell destruction. No hemosiderosis was seen in the liver or kidneys of any animal.

The only other hematological abnormality was an increase in platelet counts at the 1600-ppm dose in both sexes.

D. We assess that the only toxicologically important clinical chemistry alteration was the increase in alkaline phosphatase at the 800- and 1600-ppm doses in both sexes. This correlated with histopathologic finding of centrilobular or midzonal hepatocellular hypertrophy. SGPT values were statistically lower in the dosed female animals as compared to controls. Random fluctuations in BUN, cholesterol, phosphorus, creatinine, and calcium were not considered of toxicological importance, but resulted from the small sample size.

E. No gross pathologic alterations were observed at necropsy that could be associated with administration of RH-3866. The absolute liver weights of the 800- and 1600-ppm male and female dogs were significantly higher than the controls. The relative liver weights were also greater in both sexes at 1600 ppm and in the males at 800 ppm. These findings correlate with the histopathologic alterations observed in the liver in male dogs administered 200 ppm and above and in female dogs administered 800 ppm and above. The incidence and severity of chronic interstitial nephritis in males were increased in the two highest dose groups. This may have been compound related but an unequivocal conclusion cannot be reached due to the minimum effect and the spontaneous frequency of this type of lesion in dogs.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. A-8; Appendix B, Protocol, CBI pp. A2-A18; and Appendix C, CBI Table 1, Body Weight, pp. T-2 to T-5, and CBI Table 2, Food Consumption, pp. T-6 to T-9.

APPENDIX A

Materials and Methods

MYCLOBUTANIL 128857

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Pages 23 through 58 are not included.

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004937

EPA: 68-02-4225
TASK: 033-05
October 31, 1985

DATA EVALUATION RECORD

RH-3866

Mutagenicity--In Vivo Cytogenetic Study in Mice

STUDY IDENTIFICATION: McLeod, P. L. and McCarthy, K. L. RH-3866 in vivo cytogenetic study in mice. (Unpublished study No. 84R-0074 prepared and submitted by Rohm and Haas, Spring House, PA; dated July 23, 1984.) Accession No. 072901.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 10-31-85

53

1. CHEMICAL: RH-3866 (alpha-butyl-alpha-4-chlorophenyl-1H-1,2,4-triazole-1-propanenitrile).

2. TEST MATERIAL: RH-3866 technical, lot No. LSPL-0016/E, TD No. 83-076, was a brown solid with a purity of 81.1 percent.

3. STUDY/ACTION TYPE: Mutagenicity--in vivo cytogenetic study in mice.

4. STUDY IDENTIFICATION: McLeod, P. L. and McCarthy, K. L. RH-3866 in vivo cytogenetic study in mice. (Unpublished study No. 84R-0074 prepared and submitted by Rohm and Haas, Spring House, PA; dated July 23, 1984.) Accession No. 072901.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E McCarroll
Date: 10-31-85

Brenda Worthy, M.T.
Independent Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 10-31-85

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I Cecil Felkner
Date: 10-31-85

Jane Harris, Ph.D.
EPA Reviewer and
Section Head

Signature: Jane E Harris
Date: 1/16/86

7. CONCLUSIONS:

A. Under the conditions of this assay, the acute (one dose) or subacute (daily x 5 days) oral exposure of male mice to the approximate maximum tolerated dose of RH-3866 (650 mg/kg) did not cause a significant increase in chromosomal aberrations in bone marrow cells sampled over the entire mitotic cycle.

B. The study was Acceptable .

Items 9 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: RH-3866, lot No. LSPL-0016/E, TD No. 83-076, is an experimental fungicide described as a brown solid; it has a purity of 81.1 percent. The test material was dispersed in corn oil; prepared solutions of the test material were based on the active ingredient (a.i.) content of the test material.

2. Test Animal: Two hundred and forty-five male CD-1 mice, weighing 9 to 13 g, were obtained from Charles River Kingston Breeding Farms, Stone Ridge, NY.

3. Animal Maintenance: Prior to initiation of the study the animals were acclimated to laboratory conditions for 13 days, which included a 6-day quarantine period. Two hundred and twenty-five animals were initially selected and randomly

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[1]Only items appropriate to this DER have been included.

distributed among wire-mesh cages in groups of five. Throughout the course of the study, animals were housed in an environment controlled for temperature (22.2-26.1°C), relative humidity (28 to 62%), and light (12 hours). With the exception of dosing intervals, animals were permitted Purina Rodent Lab Chow Checkers ad libitum; water was available ad libitum at all times.

4. Assignment to Groups: One hundred and seventy males, weighing 17.7 to 30 g, were selected from the 225 animals. Animals were randomly assigned to treatment groups, ear tagged, and identified with a unique number.

5. Compound Preparation/Dosing Procedures:

   a. Compound Preparation: Based on the active ingredient content of the test material, three concentrations were prepared as corn oil dispersions. The high-dose dispersion was achieved by melting the test material (60-63°C), stirring the liquefied test material, and dispersing it into warm corn oil. Subsequent dilutions were prepared in warm corn oil from the high-dose dispersion.

   b. Dosing Procedures: The doses of the test material used in this assay (65, 260, and 650 mg/kg) were selected to approximate dilutions of 1:40, 1:10, and 1:4, respectively, of the acute LD_{50} in CD-1 mice (2.6 g/kg). Toxicity data to support this value were furnished by the sponsor. The appropriate dose levels of the test material, at a dosing volume of 10 mL/kg, were maintained at 30-35°C with constant mixing throughout the administration period.

6. Compound Administration:

   a. Acute Cytogenetic Study: Thirty animals per group were fasted 3 hours prior to the single oral administration of the appropriate concentration of the test material or vehicle control. Animals were weighed before dosing and on days 2 and 3; toxic signs were monitored daily. Ten representative members of each group were sacrificed at 6, 24, and 48 hours after compound administration.

      The positive control, triethylenemelamine, was administered as a single dose (0.3 mg/kg, ip) to 10 animals. Animals in this group were sacrificed 24 hours post-exposure.

b. Subacute Cytogenetic Study: On each dosing day, the animals in the subacute study group were fasted 3 hours prior to treatment. Ten animals per group were orally administered a single daily dose of the appropriate concentration of the test material or vehicle control for 5 consecutive days. Animals were weighed prior to dosing, observed for toxic effects, and sacrificed 6 hours after the final dose administration.

c. Animal Sacrifice/Bone Marrow Harvest: Colchicine (1 mg/kg, ip) was injected 3 hours prior to the appropriate sacrifice interval; animals were sacrificed by cervical dislocation. Bone marrow cells were collected from both femurs by aspiration into 0.65 percent KCl. Aspirators were incubated for 15 minutes at 37°C and centrifuged; the supernatants were discarded. The pellets were fixed three times in methanol:acetic acid (3:1), pipetted onto slides, flame dried, stained, mounted, and coded.

d. Slide Analysis: A maximum of 50 well-defined metaphases per animal were scored for the presence of cytogenetic abnormalities. Chromosomal aberrations were characterized as breaks, gaps, fragments, pulverized cells, translocations, or rearrangements. Gaps were not included in the final analyses.

7. Evaluation Criteria: The data were evaluated for statistical significance ($p < 0.05$) by the Beta Binomial Model[2] and Fisher Exact Test.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Acute Cytogenetics Study: Toxic signs observed in the acute cytogenetics study following exposure to 650 mg/kg RH-3866 included lethargy (observed on day 1) and yellow-brown stains around the anogenital region and diarrhea (observed on days

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[2] Williams D. A., "The analysis of binary responses from toxicological experiments involving reproduction and teratogenicity," Biometrics 31 (1975): 949-954.

1 and 2). No toxic signs were observed on day 3 for the high-dose group. Similar signs of toxicity (yellow-brown stains around the anogenital region and diarrhea) were observed 1 day after the animals were exposed to 260 mg/kg; by day 2, all toxic signs had subsided. Evidence of a toxic effect was not seen in animals exposed to the low dose (65 mg/kg).

No statistically significant increase in chromosomal aberrations occurred after acute exposure of the male mice to 650 mg/kg RH-3866. Slides were not scored for the lower doses. Representative results are shown in Table 1.

B. Subacute Cytogenetic Study: Five consecutive daily administrations of 650 mg/kg RH-3866 resulted in overt toxicity at the high dose, similar to that seen in the acute study. Diarrhea was reported in one animal on day 1; staining around the anogenital region and lethargy persisted throughout the 5-day observation period. Piloerection was observed on day 3 in 2 of the 10 animals, and the majority of the animals exhibited piloerection on days 4 and 5 (8/10 and 7/10, respectively). Toxicological responses (lethargy and piloerection) were not evident in animals exposed to 260 mg/kg until day 5. No toxic signs were apparent in animals treated with the low dose (65 mg/kg).

No statistically significant increase in chromosomal aberrations resulted from the subacute exposure of the male mice to 650 mg/kg RH-3866. Slides were not scored for the mid and low doses. Representative results are present in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded, "When compared to vehicle treated animals, no significant increases in chromosomal aberrations were observed in the acute (single dose) or subacute (daily x 5 days) animals treated with 650 mg a.i./kg of RH-3866."

B. A quality assurance statement was present, signed, and dated July 6, 1984. The report further stated that the study was conducted according to good laboratory practices.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that this study was well conducted and the authors' interpretation of the data was correct. Toxicologic signs were evident in animals exposed to 650 mg/kg, and, based on the LD_{50}

TABLE 1. Representative Results of the Acute and Subacute *In Vivo* Cytogenetic Study in Mice with RH-3866

	Dose	Exposure[a] Time	No. of Male Animals Scored	No. of Metaphases Examined	No. of Cells with Aberrations[b]	Percent Aberrant Cells[c]
Control						
l	10 mL/kg	6h	10	449	2	0.4
		24h	10	500	3	0.6
		48h	10	460	3	0.7
		5d	10	411	4	1.0
Control						
lenemelamine	0.3 mg/kg	24h	10	392	83	21.2*
rial						
	650 mg/kg[d]	6h	10	466	2	0.4
		24h	10	486	2	0.4
		48h	10	461	2	0.4
		5d	10	468	5	1.1

er compound administration.

included.

aberrant cells = $\frac{\text{No. of cells with aberrations}}{\text{No. of metaphases examined}} \times 100$

gns observed at all observation intervals.

antly different from 24-hour control value at $p < 0.05$ by Fischer Exact Test and Beta Binomial Model.

omosome preparations for the 65 and 260 mg/kg dosing groups were not scored.

data, 650 mg/kg approximated the maximum tolerated dose.

The statistically significant increase in chromosomal aberrations in mice treated with the positive control (triethylenemelamine, 0.3 mg/kg, ip) adequately demonstrated the sensitivity of the test system to detect clastogenic agents.

Item 15--see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods, CBI pp. 2-7; Protocol, CBI pp. 13-22.

APPENDIX A

Materials and Methods - CBI pp. 2-7
Protocol - CBI pp. 13-22

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EPA: 68-01-6561
TASK: 12303
September 13, 1985

DATA EVALUATION RECORD

RH-53,866

Mutagenicity--Reverse Mutation in Salmonella

STUDY IDENTIFICATION: Chism, E. M. RH-53,866; microbial mutagen test. (Unpublished study No. 83R 0246 prepared and submitted by Rohm and Haas, Spring House, PA; dated January 31, 1984.) Accession No. 072901.

APPROVED BY:

L. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: L. Cecil Felkner

Date: 9-13-85

1. CHEMICAL: RH-53,866 (alpha-butyl-alpha-4-chlorophenyl-1H-1,2,4-triazole-1-propanenitrile).

2. TEST MATERIAL: RH-53,866 technical, lot No. LAP-0298, TD No. 83-260, contained 90.4 percent active ingredient.

3. STUDY/ACTION TYPE: Mutagenicity--reverse mutation in Salmonella.

4. STUDY IDENTIFICATION: Chism, E. M. RH-53,866; microbial mutagen test. (Unpublished study No. 83R 0246 prepared and submitted by Rohm and Haas, Spring House, PA; dated January 31, 1984.) Accession No. 07[illegible]901.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E McCarroll
Date: 9-13-85

Brenda Worthy, M.T.
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Dynamac Corporation

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6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
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Dynamac Corporation

Signature: I Cecil Felkner
Date: 9-13-85

Jane Harris, Ph.D.
EPA Reviewer and
Section Head

Signature: Jane E Harris
Date: 1/16/86

7. CONCLUSIONS:

A. Under the conditions of this assay, RH-53,866 technical, lot No. LAP-0298, TD No. 83-260, did not cause an appreciable increase in the reversion to histidine prototrophy of four S. typhimurium strains at doses ranging from 75 to 7500 µg/plate with or without S9 activation.

B. The study was Acceptable.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details).

1. The test material, RH-53,866 technical, lot No. LAP-0298, TD No. 83-260, was described as a triazole containing 90.4 percent active ingredient. The test material was stored at room temperature and dissolved in dimethylsulfoxide (DMSO).

2. S. typhimurium strains TA1535, TA1537, TA98, and TA100 were obtained from Dr. Bruce Ames, University of California, Berkeley. The strains were maintained at -80°F in nutrient broth-DMSO. Late logarithmic phase cultures, generated from the frozen stocks, were used for this assay.

3. The S9 fraction used for metabolic activation was prepared in accordance with the method of Ames et al.[2] and was derived from the livers of Charles River COBS-CD-Br rats induced with Aroclor 1254.

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[1] Only items appropriate to this DER have been included.

[2] Bruce N. Ames, Joyce McCann, and Edith Yamasaki. "Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test." Mutation Research 31 (1975): 347-364.

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4. Mutagenicity Assay: One tenth of the appropriate concentration of the test material, which covered a 3- to 4-log range, and 0.5 mL of 0.1 M phosphate buffer mix (pH 7.4) with or without 0.5 mL of S9 mix for activation were added to sterile culture tubes. Two milliliters of 0.7 percent unsupplemented agar were added to each reaction mixture; the contents of each tube were mixed and poured over the surface of triplicate minimal plates (1.4 µg/mL L-histidine HCl, 1.65 µg/mL biotin in 15 mL Vogel-Bonner agar) and single supplemented plates containing 100 µg/mL histidine and 1.65 µg/mL biotin. The negative and positive controls were treated in a similar manner. Plates were incubated at 37°C for 72 hours. Following incubation, the revertant colonies per plate were scored.

5. Evaluation Criteria: The results were considered significant if a difference ($p = 0.05$) between the mean number of revertants on the test plates and the mean number of spontaneous revertants on the control plates was calculated.

6. The data were analyzed in accordance with the method of Mohn and Ellenberger.[3]

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Mutagenicity Assay: The five doses (75, 250, 750, 2500, and 7500 µg/plate) selected for the mutagenicity assay were tested in the presence and absence of S9 activation. At the highest dose of the test material (7500 µg/plate), cytotoxicity, as indicated by a marked reduction in revertant colonies, was seen under S9-activated and nonactivated conditions. In the presence of S9 activation, cytotoxicity was apparent for strains TA1535, TA1537, and TA100 at a dose of 2500 µg/plate. No appreciable increase in his^+ colonies of any strain was observed at the remaining noncytotoxic doses either with or without S9 activation. Representative data for all strains are presented in Table 1.

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[3]Mohn, G. R. and J. Ellenberger. "The use of Escherichia coli K12/343/113 (λ) as a multi-purpose indicator strain in various mutagenicity testing procedures," in Handbook of Mutagenicity Test Procedures, Kilbey, Legator, Nichols and Ramel, eds. (Amsterdam: Elsevier Scientific Publishing Co., 1977), pp. 95-118.

TABLE 1. Representative Results of the S. typhimurium Mutagenicity Assay with RH-57,866

Substance	S9 Activation	Dose (µg/plate)	Revertants per Plate of Bacterial Tester Strain TA1535	TA1537	TA98	TA100
Solvent Control[a]						
DMSO	-		32.3±6.4	11.9±7.1	23.3±6.4	101.3±12.8
	+		38.8±5.5	35.2±6.8	59.7±9.3	103.5±15.6
Positive Controls[b]						
2-Anthramine	+	10	441.3	231.0	—	2240.3
2-Acetylaminofluorene	+	50	—	—	1012.8	—
Test Material[b]						
RH-57,866	-	2500[c]	36.0	4.3	25.3	109.3
	+	750[c]	23.3	22.7	67.3	107.3
	-	7500[d]	12.3	0.7	16.0	33.3
	+	7500	0	0	0	0

[a] Mean and S.D. of counts.

[b] Means reported by authors.

[c] Highest noncytotoxic dose; at doses below these levels (i.e., 75, 250 or 750 µg/plate), revertant counts were comparable to the solvent.

[d] Highest test dose.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The author concluded, "RH-53,866 (lot No. LAP-0298) did not demonstrate mutagenic activity when tested following our standard protocol 83P-046."

B. A quality assurance statement was present, signed, and dated January 30, 1984. The report indicated that the study was conducted in compliance with generally recognized, currently good laboratory practices.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

It is our assessment that the author's interpretation of the data was correct. The mean spontaneous reversion frequencies for the solvent control (DMSO) were presented with standard deviations and were within acceptable ranges for a 72-hour incubation.[5] The response of strains TA1535, TA1537, and TA100 to 2-anthramine (10 µg/plate) and strain TA98 to 2-acetylamenofluorene (50 µg/plate) adequately measured the sensitivity of these strains to detect the mutagenic metabolites of the appropriate promutagen.

Item 15--see footnote 1.

16. CBI APPENDIX:

Appendix A, Materials and Methods (Protocol), CBI pp. 2-6.

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[4]deSerres, Frederick J. and Michael D. Shelby, "Recommendations on data production and analysis using the Salmonella microsomal mutagenicity assay." Mutation Research 64 (1979): 159-165.

[5]Ibid.

APPENDIX A

Materials and Methods (Protocol)

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

1. CHEMICAL: RH-53,866; alpha-butyl-alpha-4-chlorophenyl-1H-1,2,4-triazole-1-propanenitrile.

2. TEST MATERIAL: RH-53,866 technical, lot No. LSPL 0016/E, was described as a brown solid with a purity of 81.1%.

3. STUDY/ACTION TYPE: CHO/HGPRT point mutation assay.

4. STUDY IDENTIFICATION: O'Neill, P. J., Foxall, S. and Byers, M. J. RH-53,866 technical CHO/HGPRT point mutation assay. (Unpublished study No. 84R-046 prepared and submitted by Rohm and Haas Company, Spring House, PA; dated May 29, 1984.) Accession No. 072901.

5. REVIEWED BY:

Brenda Worthy, M.T.
Principal Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 1-24-86

Nancy E. McCarroll, B.S.
Independent Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 1-24-86

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 1-24-86

Jane Harris, Ph.D.
EPA Reviewer and
Section Head

Signature:
Date: 1/24/86

7. CONCLUSIONS:

A. Under the conditions of the assay, RH-53,866 did not induce a mutagenic response in the CHO/HGPRT point mutation assay at non-activated doses ranging from 25 to 90 µg/mL. The results of the S9-activated assays were more difficult to interpret. Contamination as well as variations in cytotoxicity and compound precipitation affected the reproducibility of the assays, thus precluding the accurate assessment of the three individual S9-activated tests. Collectively, however, no qualitative evidence of a mutagenic response was uncovered at doses up to 175 µg/mL, the highest cytotoxic dose tested in the presence of S9 activation. The positive controls, ethylmethanesulfonate (EMS) and 7,12-dimethylbenzanthracene (DMBA) caused a significant increase in mutation frequency (MF) compared to the solvent control, demonstrating the sensitivity of the target cells.

B. The study is acceptable.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: RH-53,866 technical, lot No. LSPL 0016/E, was described as a brown solid with a purity of 81.1%. The test material was warmed at 55 to 60°C until liquified and diluted in dimethylsulfoxide (DMSO), the solvent control.

2. Cell Line: The Chinese hamster ovary (CHO) cell used in this study was the BH_4 subclone of the $CHO\text{-}K_1$ cell line developed by Dr. Abraham Hsie. Stock cultures were maintained in liquid nitrogen. Growing cultures were periodically analyzed for mycoplasma contamination, karyotype stability, 6-thioguanine (6-TG) sensitivity, and aminopterin resistance.

3. S9 Fraction: The S9 fraction was prepared from the livers of male Sprague-Dawley rats induced with Aroclor 1254.

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[1]Only items appropriate to this DER have been included.

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4. Preliminary Cytotoxicity Assay: Cultures, seeded at 5×10^5 cells/plate, were exposed to an unspecified number of test material doses over at least a 4-log concentration for 5 hours with S9 activation or 18 to 20 hours without activation. Cells were also treated with the solvent or positive controls. Two days after seeding, cells were subcultured with fresh growth medium (hypoxanthine free), and cytotoxicity was determined by the plating efficiency of the test material relative to the solvent control.

5. CHO Assay: Based on the cytotoxicity data, at least four concentrations were selected for the CHO assay performed with or without S9 activation. Doses were selected to span a toxicity range of 10 to 90% survival. Doses for repeat trials were selected based on the results of the initial trial.

   a. Treatment: Cells were treated with the appropriate level of test material solvent or positive control for 5 hours with S9 activation or 18-20 hours without S9 activation at 37°C. Cultures were washed with saline to end exposure to the test material. For cytotoxicity assessment, 200 cells were plated and the remaining cells, seeded at a density of 1×10^6, were subcultured for the mutation expression period.

   b. Mutation Expression Period: Cells used for mutant expression were maintained in logarithmic growth by subculturing twice during the 8-day expression period.

   c. Mutant Selection: Selection of 6-thioguanine-resistant mutants (6-TG^r) was accomplished by plating 2×10^5 cells (five replicates) from each treatment group into media containing 10 µM 6-TG. Cell survival (at selection) for each treatment group was assessed from four plates, seeded with 200 cells/plate in medium free of 6-TG. Selection and survival plates were incubated for 7 days, fixed, stained, and counted. MF was calculated as the number of 6-TG^r mutants/10^6 survivors.

6. Evaluation Criteria: The test material was considered positive if there was a significant and reproducible dose-related increase in MF relative to the solvent control. If an increase in the MF occurred at one dose level, then the result should be reproduced in an independent assay.

7. Statistical Analysis: MF data were transformed and analyzed by methods similar to those of Snee and Irr.[2] Analysis of variance was assessed by Duncan's multiple range test.

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[2] Snee, R. D. and Irr, J. D. Design of a statistical method for analysis of mutagenesis at the hypoxanthine-guanine phosphoribosyl transferase locus of cultured Chinese hamster ovary cells. Mutat. Res. (1981) 85: 77-98.

B. Protocol: See Appendix B.

12. REPORTED RESULTS: The results of duplicate cultures were reported separately and referred to as replicas 1 or 2. Where expedient, the results were averaged by the reviewers.

A. A CHO Mutation Assay--Without S9 Activation:

1. Cytotoxicity Study: RH-53,866 was assayed at nine doses ranging from 0.5 to 100 µg/mL. Cytotoxicity results, as assessed by plating efficiency, ranged from 11.9% survival at 100 µg/mL to 89.9% survival at 5 µg/mL.

2. Mutation Assay #1: Based on the cytotoxicity results, four doses of the test material (25, 60, 80, and 100 µg/mL) were chosen for the mutation assay. Due to cytotoxicity, the cultures treated with 100 µg/mL were terminated prior to selection. Average survival for the remaining levels was 37% at 80 µg/mL and 104% at 25 µg/mL. The MF of the solvent control (average of two replicate analyses) was 9.1/6-TG^r mutants/10^6 survivors. The average MF of the positive control, EMS dosed at 100 nL/mL, was 725.5 mutants/10^6 survivors. Treatment with the test material resulted in average MFs of 49.1, 3.., and 14.7 mutants/10^6 survivors at dose levels of 80, 60, and 25 µg/mL, respectively.

A significant increase in MF was observed at the 80-µg/mL dose; however, the average plating efficiency for the DMSO control cultures was low (57%); therefore, a repeat analysis (#2) was performed.

Mutation Assay #2: This assay was conducted with doses of 60, 80, 85, and 90 µg/mL of the test material. Average cell survival ranged from 88% at the 60-µg/mL dose to 27% at the 90-µg/mL dose.

The average MF for the solvent and positive (EMS) control were 7.2 and 544.2/10^6 survivors, respectively. The average MFs of the test material at doses of 60, 80, 85, and 90 µg/mL were 0, 4.5, 1.5, and 0.7 per 10^6 survivors, respectively.

No increase in MF was observed at the doses tested when compared to the solvent control; therefore, the increase noted at the 80-µg/mL dose in the initial assay was neither confirmed nor considered treatment related (see Table 1 for representative results from mutation assay #2).

TABLE 1. Representative Results from CHO Mutation Assay with RH-53,866—without S9 Activation[a]

Substance	Dose/mL	Toxicity Post-Treatment (% Survival)	Plating Efficiency At Selection (%)	Average Mutant Colonies per plate	6-TG^r Mutants/10^6 Survivors[b]
Solvent Control					
DMSO	0.5%	100.0	76.1	1.1	7.2
Positive Control					
EMS	100 nL	66.5	53.0	57.6	544.2*
Test Material					
RH-53,866	60 µg[c]	88.0	67.1	0	0
	90 µg[d]	27.0	71.2	0.1	0.7

[a]Results from replicas 1 and 2, averaged by our reviewers.

[b]Mutant frequency/10^6 = $\frac{\text{Mutant colonies/plate}}{\text{Plating efficiency}/100 \times 2 \times 10^5} \times 10^6$

[c]Lowest dose tested.

[d]Highest dose tested (doses at 80 and 85 µg/mL were comparable to solvent control).

*Significant increase as reported by the author.

B. CHO Mutation Assay--with S9 Activation:

1. Cytotoxicity Study: An initial cytotoxicity assay was performed with eight doses of the test material ranging from 1 to 1000 µg/mL with S9. No cells survived at the two highest dose levels, 500 and 1000 µg/mL. Precipitation of the test material was observed at these concentrations. Plating efficiency (cell recovery) ranged from 81.2% at 1 µg/mL to 90.2% at 100 µg/mL.

   A second cytotoxicity assay was conducted with six doses ranging from 175 to 500 µg/mL. The test material proved lethal at the four highest doses (300, 350, 400, and 500 µg/mL) with 0.3% recovery at 200 µg/mL. Precipitation was also noted above 200 µg/mL. The lowest dose (175 µg/mL) resulted in a 48.2% recovery.

   In a third cytotoxicity assay using nine doses of the test material ranging from 100 to 200 µg/mL, lethality was observed at the three highest doses, 180, 190, and 200 µg/mL. Treatment with 150, 160, and 170 µg/mL resulted in 39.4, 7.8, and 1.1% cell recovery. At the three lowest doses, 100, 120, and 140 µg/mL, recovery was >60%. No precipitation was reported at any dose level.

   In the final cytotoxicity assay, cells were treated with seven doses of the test material ranging from 120 to 200 µg/mL. At the three highest doses, 160, 170, and 200 µg/mL, plating efficiency ranged between 39.0 to 0%; precipitation was noted at these concentrations. Recovery at the four lower doses, 120, 135, 150, and 155 µg/mL, ranged from 59 to 48.9%.

2. Mutation Assay #1: Based on the cytotoxicity data, RH-53,866 was tested with S9 activation at doses of 120, 150, 155, 160, and 170 µg/mL in the mutation assay. The assay was performed with replica cultures; replica 1 was lost due to contamination. Cell survival in the remaining replica cultures ranged from 100 to 1%, with an MF range of 13.6 to 23.8/10^6 survivors at the 160-µg/mL dose. The test material was toxic at the highest dose. The assay was repeated (#2) because the dose range was inadequate.

3. Mutation Assay #2: The test material was assayed at doses of 120, 150, 155, and 160 µg/mL in replicate. Replica 2 was lost due to contamination. Survival in the remaining cultures ranged from 82% at 120 µg/mL to 6% at the 160-µg/mL dose. Due to cytotoxicity at the two highest doses, the MF could not be assessed; however, at the two lower doses (120 and 150 µg/mL) the MFs were 2.9 and 0 mutants/10^6 survivors, respectively. The assay was repeated (#3).

4. Mutation Assay #3: The test material was assayed at doses of 165, 170, and 175 µg/mL. The survival range was 121 to 19%. In replica 2, survival at the 175 µg/mL dose was 249%; this was considered an error in dilution. The average MFs (averaged by our reviewers from the replicates) were 3.7 and 3.1 mutants/10^6 survivors at doses of 165 and 170 µg/mL, respectively; the mutant frequency was 0/10^6 survivors at 175 µg/mL in replica 1; replica 2 at 175 µg/mL was terminated due to contamination.

   The solvent control MF, averaged by study authors, was 20.7/10^6. The positive control MF (7 µg/mL DMBA) was 236.7 mutants/10^6 survivors (replica 1 was lost due to contamination).

   No increase in the MF was observed when cells were dosed with the test material at concentrations ranging from < 25 to >75% cell survival and with metabolic activation using 1 mg S9 protein/mL (see Table 2 for representative results).

5. Additional Studies with Alternative S9 Concentrations: In addition to treatment of the test material with S9 at 1 mg protein/mL, the test material was assayed at 160 µg/mL with 0.3 and 2 mg S9 protein/mL. Cells treated at 160 µg/mL with 2 mg protein/mL S9 resulted in an MF of 1 mutant/10^6 survivors compared to the solvent control MF of 6.6 mutants/10^6 survivors. In the presence of 0.3 mg protein/mL S9, this dose level resulted in a cell survival of < 5%; the assay was terminated. Insufficient data were reported for both S9 concentrations; these results are, therefore, not presented in tabular form.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "RH-53,866 did not induce mutation at the HGPRT locus in CHO cells when tested from 25 to 90 µg/mL without metabolic activation. These treatments resulted in 104% to 23% cell survival. With a metabolic activation system RH-53,866 did not induce mutations when tested from 120 to 175 µg/mL, which yielded 100% to 19% cell survival, respectively."

B. A quality assurance statement was presented and signed.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. It is our assessment that the authors interpreted the data correctly, and that RH-53,866 did not induce an increase in mutation frequency in Chinese hamster ovary (CHO) cells at the HGPRT locus with or without S9 activation.

TABLE 2. Representative Results from CHO Mutation Assay with RH-53,866--with S9 Activation[a]

Substance	Dose/mL	Toxicity Post-Treatment % Survival	Plating Efficiency At Selection (%)	Mutant Colonies per plate	Mutant Frequency/ 10^6 Survivors
Solvent Control DMSO[b]	0.83%	93	72.9	0	0
Positive Control DMBA[b]	7 µg	108	71.6	32.2	224.9*
Test Material RH-53,866[b]	120 µg	82	69.1	0.4	2.9
Solvent Control DMSO[c]	0.83%	81	94	6.6	35.1
Positive Control DMBA[d]	7 µg	106	73.5	34.8	236.7*
Test Material RH-53,866[c]	175 µg	43	74.6	0	0

[a] S9 at 1 mg protein/mL.

[b] Mutation assay #2 results for lowest dose tested and controls from Table IX, CBI (p. 27), replica 1; all results for replica 2 were lost due to contamination.

[c] Mutation assay #3 results for highest dose tested and solvent control from Table X, CBI (p. 28), replica 1.

[d] Mutation assay #3 results for positive control from Table X, CBI (p. 28), replica 2; results for replica 1 were lost due to contamination.

The results of the nonactivated assays were straightforward and showed that RH-53,866 assayed within a cytotoxic range did not cause an increase in the MF. A significant increase in the MF was reported at 80 µg/mL in the first assay; however, this was not reproduced in a confirmation test when a narrower range of doses, clustered around the 80-µg/mL dose, was tested.

For various technical reasons (precipitation, excessive cytotoxicity, noncytotoxicity or contamination) the study authors performed three S9-activated assays. None of the three assays represented a completely successful experiment; nevertheless, the data supported the authors' conclusion that the test material was not mutagenic. However, the effects observed in the cytotoxicity and precipitation data suggest to us that the solubility characteristics of RH-53,866 may have contributed to the erratic results obtained in the S9-activated assays. RH-53,866, a brown solid substance was liquified at 55-60°C before dilutions were made with DMSO. It is conceivable that the test material recrystallized in the presence of S9-activation which resulted in erratic exposure of the cells to minute particles of the test material throughout the 7-day incubation period. Hence, reproducibility of the assay was affected, and accurate assessment of the assays was not possible. However, a qualitative interpretation could be made, and RH-53,866 did not elicit a mutagenic response in the presence of S9 activation.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 1-12, and Appendix B, Protocol, CBI pp. 31-44.

APPENDIX A

Material and Methods

MYCLOBUTANIL 128857

Page_____ is not included in this copy.

Pages 107 through 133 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

004937

EPA: 68-01-6561
TASK: 123
August 1, 1985

DATA EVALUATION RECORD

RH-3866

Acute Oral Toxicity Study in Rats

STUDY IDENTIFICATION: Krzywicki, K. M. and Morrison, R. D. Acute definitive oral LD_{50}. (Unpublished study No. 84-063 A & B by Rohm and Haas Company, Toxicology Department, Spring House, PA, for the Rohm and Haas Company, Spring House, PA; dated July 19, 1984.) Accession No. 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: James R. Felkner IV

Date: 8-1-85

004937

1. CHEMICAL: RH-3866.

2. TEST MATERIAL: RH-53,866 Technical; TD Nos. 84-006 and 84-064; Lot 83159-5; 2-Butyl-2-(4-chlorophenyl)-1H-1,2,4-triazole-1-propane-nitrile, 91.9% Technical.

3. STUDY/ACTION TYPE: Acute Oral Toxicity Study in Rats.

4. STUDY IDENTIFICATION: Krzywicki, K. M. and Morrison, R. D. Acute oral LD_{50}. (Unpublished study No. 84-063 A & B by Rohm and Haas Company, Toxicology Department, Spring House, PA, for the Rohm and Haas Company, Spring House, PA; dated July 19, 1984.) Accession No. 072896.

5. REVIEWED BY:

Barry E. O'Keefe, M.S.
Principal Reviewer
Dynamac Corporation

Signature: Barry E. O'Keefe
Date: 8-1-85

Sharon M. Ambrose, B.S.
Independent Reviewer
Dynamac Corporation

Signature: Sharon M. Ambrose
Date: 8-1-85

6. APPROVED BY:

Finis Cavender, Ph.D.
Acute Toxicology
Technical Quality Control
Dynamac Corporation

Signature: Finis Cavender
Date: 8/1/85

~~Irving Mauer, Ph.D.~~
~~EPA Reviewer~~

Signature: _____
Date: _____

Jane E. Harris, Ph.D.
EPA Section Head

Signature: Jane E. Harris
Date: 8/2/85

7. SUMMARY:

Groups of twenty male and twenty female CRCD rats were gavaged with a single oral dose of RH-53,866 in a corn oil vehicle (10 ml/kg) at 0, 0.75, 1.04 (only 10 animals/sex), 1.05 (only 10 animals/sex), 1.34, 1.82, and 2.41 (only 15 animals/sex) g/kg of body weight. Animals were observed for 14 days and then sacrificed. Several deleterious signs, e.g., passiveness, ataxia, scant droppings, etc., were observed at doses $\geq$ 0.75 g/kg for both males and females. The day 14 mortalities were as follows:

Dosage (g/kg)	Deaths at Day 14 (Died/Dosed) Males	Females
0	0/20	0/20
0.75	0/20	1/20
1.04/1.05	6/20	2/20
1.34	10/20	4/20
1.82	13/20	8/20
2.41	9/15	7/15

For rats that died, prominent observations at necropsy included red or brown stained muzzle, yellow or brown stained anogenital area, and a slight reddening of the lungs. At necropsy, for surviving rats, a few cases of alopecia were noted. Based on these data, the acute definitive oral LD_{50} for RH-53,866 in male rats was calculated to be 1.60 g/kg (confidence limits not given) and in female rats 2.29 g/kg (range: 1.82-4.14 g/kg).

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

This study was conducted in an acceptable manner. The acute oral LD_{50} of RH-53,866 in male rats is 1.60 g/kg and in female rats is 2.29 g/kg, which correspond to Toxicity Category III.

A quality assurance statement was not present in this report.

9. CLASSIFICATION:

Core Classification: Minimum.

Toxicity Category: III.

Acute Oral LD_{50} : 1.60 g/kg males.
2.29 g/kg females.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

004937

EPA: 68-01-6561
TASK: 123
August 1, 1985

DATA EVALUATION RECORD

RH-3866

Acute Oral Toxicity Study in Mice

STUDY IDENTIFICATION: Morrison, R. D. Acute definitive oral LD_{50}. (Unpublished study No. 84R-0153 by Rohm and Haas Company, Toxicology Department, Spring House, PA, for the Rohm and Haas Company, Spring House, PA; dated August 3, 1984.) Accession No. 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: James A. Hunt for

Date: 8-1-85

1. CHEMICAL: RH-3866.

2. TEST MATERIAL: RH-53,866 Technical; TD No. 84-141; Lot 83159-5; 2-Butyl-2-(4-chlorophenyl)-1H-1,2,4-triazole-1-propane-nitrile, 91.9% Technical.

3. STUDY/ACTION TYPE: Acute Oral Toxicity Study in Mice.

4. STUDY IDENTIFICATION: Morrison, R. D. Acute definitive oral LD_{50}. (Unpublished study No. 84R-0153 by Rohm and Haas Company, Toxicology Department, Spring House, PA, for the Rohm and Haas Company, Spring House, PA; dated August 3, 1984.) Accession No. 072896.

5. REVIEWED BY:

Barry E. O'Keefe, M.S.
Principal Reviewer
Dynamac Corporation

Signature: Barry E O'Keefe
Date: 8-1-85

Sharon M. Ambrose, B.S.
Independent Reviewer
Dynamac Corporation

Signature: Sharon M Ambrose
Date: 8-1-85

6. APPROVED BY:

Finis Cavender, Ph.D.
Acute Toxicology
Technical Quality Control
Dynamac Corporation

Signature: Finis Cavender
Date: 8/1/85

~~Irving Mauer, Ph.D.~~
~~EPA Reviewer~~

Signature: _____
Date: _____

Jane E. Harris, Ph.D.
EPA Section Head

Signature: Jane E Harris
Date: 8/2/85

7. SUMMARY:

Ten male CD-1 mice were gavaged with a single oral dose of RH-53,866 in a corn oil vehicle (10 ml/kg; warmed to approximately 38°C) at 0, 1.14, 1.76, 2.83, or 4 42 g/kg of body weight. Animals were observed for 14 days, and then sacrificed. The only prominent toxic signs observed were passiveness, ataxia, and brown or yellow stained anogenital areas, with a positive dose response from 0 to 2.83 g/kg. A few animals died within two days of treatment; one at each dose level tested. For mice that died, necropsy observations included red stained muzzle, yellow stained anogenital areas, reddened lungs, and red-fluid filled stomachs. At necropsy for surviving mice, no gross changes were observed. Based on these data, the oral LD_{50} for RH-53,866 in mice is greater than 4.42 g/kg.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

This study was conducted in an acceptable manner. The acute oral LD_{50} of RH-53,866 in male mice is greater than 4.42 g/kg, which corresponds to Toxicity Category III.

A quality assurance statement was not present in this report.

9. CLASSIFICATION:

Core Classification: Supplementary.

Toxicity Category: III.

Acute Oral LD_{50}: > 4.42 g/kg

004937

EPA: 68-01-6561
TASK: 123A-3
September 6, 1985

DATA EVALUATION RECORD

RH-53,866

Acute Dermal Toxicity Study in Rabbits

STUDY IDENTIFICATION: Krzywicki, K. M. Acute dermal toxicity study in New Zealand white rabbits. (Unpublished TD report No. 84R-134A and 134B prepared and submitted by Toxicology Department, Rohm and Haas, Spring House, PA 19447; dated July 30, 1984.) Accession No. 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 9-6-85

1. CHEMICAL: RH-53,866 [α-butyl-α-(4-chloro-phenyl)-1H-1,2,4-triazole 1-propanenitrile].

2. TEST MATERIAL: RH-53,866, technical grade, was of 91.9 percent purity and was described as a yellow solid. The test sample was TD No. 84-141, lot No. 83159-5.

3. STUDY/ACTION TYPE: Acute dermal toxicity study in rabbits.

4. STUDY IDENTIFICATION: Krzywicki, K. M. Acute dermal toxicity study in New Zealand white rabbits. (Unpublished TD report No. 84R-134A and 134B prepared by Toxicology Department, Rohm and Haas, Spring House, PA 19447; dated July 30, 1984.) Accession No. 072896.

5. REVIEWED BY:

William M. Butler, M.S.
Principal Reviewer
Dynamac Corporation

Signature: William M. Butler
Date: 9-6-85

Patricia A. Turck, M.S.
Independent Reviewer
Dynamac Corporation

Signature: Patricia A. Turck
Date: 9/6/85

6. APPROVED BY:

Finis Cavender, Ph.D.
Acute Toxicity
Technical Quality Control
Dynamac Corporation

Signature: Finis Cavender
Date: 9/6/85

Jane Harris, Ph.D.
EPA Reviewer and Section Head

Signature: Jane E Harris
Date: 9/7/85

7. SUMMARY:

Six male and six female New Zealand white rabbits were used in this study. They were obtained from Hazleton Dutchland, Denver, PA, quarantined, and acclimatized to laboratory conditions for at least 6 days. Twenty hours before dosing, the backs of all animals were closely clipped free of hair with electric clippers. Care was taken to avoid abrading the skin. Individual animal body weight was not reported. The test material was administered by topical application in a dosage of 5 g/kg.

The test material was a yellow solid; therefore, it was warmed to 80°C and then cooled to 35°C before topical application as a viscous liquid. The material was spread over the skin in a thin uniform film and held in place at the application site with an impervious cuff. Twenty-four hours after application, the impervious cuffs were removed and the application sites were wiped gently with paper tissue. The test substance was not removed by wiping, hence all of the applied dose remained on the skin. All of the rabbits survived the 14-day study.

All of the rabbits were subsequently observed for preening of areas of the treated skin and possibly ingesting the test substance. One male rabbit showed signs of systemic toxicity (e.g., red stains on drop sheet, scant droppings, brown-stained anogenital and genital areas from days 5 to 8). The remaining five males and all six female rabbits showed no signs of systemic toxicity for the duration of the experiment. The one rabbit showing signs of toxicity recovered on day 8 and was normal for the duration of the study.

Signs of skin irritation were visible in both male and female animals on day 1, including moderate to severe erythema that persisted for the duration of the study and slight edema that persisted to day 6 in male rabbits and to day 11 in female rabbits. Eschar was observed in both sexes on day 4 and lasted until day 12 in the female rabbits and to the end of the study in male rabbits. Desiccation was observed on day 4 in male rabbits and on day 7 in female rabbits, and it persisted in both sexes until the end of the study.

At termination of the study, a gross necropsy was performed; 1/6 males showed small pinpoint areas of eschar formation at the application site. The remaining 5/6 males and all 6/6 females showed no gross changes.

The authors concluded that the test substance was practically nontoxic to male and female rabbits by a single dermal application. The dermal LD_{50} was greater than 5 g/kg in the rabbit.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The design and conduct of the study were generally acceptable. The observation that the test material could not be wiped from the animals' skin meant that a single, 24-hour dermal application became a continuous 14-day exposure to the chemical. This could account for eschar and desiccation being observed at the application site. If the guideline procedure had been used (if the test material had been applied to gauze pads and then held loosely in contact with the skin), the bonding or sticking that was reported may not have occurred. We agree with the author that the test material was practically nontoxic in a single dermal application. The eschar, desiccation, and irritation observed may be the result of the continuous bonding to the skin.

There was no quality assurance statement present.

9. CLASSIFICATION:

Core Classification: Minimum.

Toxicity Category: III.

Dermal LD_{50}: >5 g/kg for both male and female rabbits.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

004937

EPA: 68-01-6561
TASK: 123
September 3, 1985

DATA EVALUATION RECORD

RH-53,866

Primary Dermal Irritation Study in Rabbits

STUDY IDENTIFICATION: Krzywicki, K. M. and Bonin, R. Primary skin irritation study in rabbits. (Unpublished report No. 84R-134A prepared and submitted by Rohm and Haas, Spring House, PA; 19477), dated August 3, 1984.) Accession No. 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 9-3-85

1. CHEMICAL: RH-53,866; 2-butyl-2-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile.

2. TEST MATERIAL: RH-53,866, technical grade, lot No. 83159-5, a yellow viscous liquid/solid, consisted of 91.9 percent active ingredient.

3. STUDY/ACTION TYPE: Primary dermal irritation study in rabbits.

4. STUDY IDENTIFICATION: Krzywicki, K. M. and Bonin, R. Primary skin irritation study in rabbits. (Unpublished report No. 84R-134A prepared and submitted by Rohm and Haas, Spring House, PA; dated August 3, 1984.) Accession No. 072896.

5. REVIEWED BY:

William M. Butler, M.S.
Principal Reviewer
Dynamac Corporation

Signature: William M. Butler
Date: 8-30-85

Peggy Perreault, B.S.
Independent Reviewer
Dynamac Corporation

Signature: Peggy Perreault
Date: 8-30-85

6. APPROVED BY:

Robert Weir, Ph.D.
Acute Toxicology
Technical Quality Control
Dynamac Corporation

Signature: Robert Weir
Date: 8-30-85

Jane Harris, Ph.D.
EPA Reviewer and
Section Head

Signature: Jane C. Harris
Date: 8/31/85

7. SUMMARY:

A. Six male New Zealand white rabbits were used in this study. The rabbits were obtained from Hazelton Dutchland, Denver, PA; the rabbits weighed between 2.0 to 3.5 kg. The animals were acclimated to laboratory conditions for 6 days and were presented with rabbit chow and water ad libitum. Approximately 24 hours prior to dose application, the hair on the dorsal area of the trunk was closely clipped with a small animal electric clipper. Care was taken to avoid abrading the skin.

The test material, which was a yellow solid, was warmed to 80°C to liquify, and then cooled to approximately 28°C. A 0.5 ml dose of the test material, a new viscous liquid, was placed on a gauze patch and applied to the shaved skin of each rabbit. The patches were covered and held in continuous contact with the skin by impervious cuffs for 4 hours. After the 4-hour exposure, the patches and cuffs were removed, and the application sites were wiped gently. Skin reactions were evaluated 1, 24, and 72 hours and 7 days after exposure to the test material.

The skin of the rabbits did not show any signs of irritation, erythema, or edema. The 72-hour mean irritation score was 0.0. Based on the mean irritation score the test substance was practically nonirritating to the skin of rabbits.

8. REVIEWER'S COMMENTS AND QUALITY ASSURANCE MEASURES:

A. The design, conduct, and reporting of this study is acceptable.

A quality assurance statement was not incluced; however, the protocol indicated that the study should have been performed according to good laboratory practices.

9. CLASSIFICATION:

Core Classification: Minimum.

Toxicity Category: IV.

The 72-hour mean irritation score equaled 0.0, RH52,866 was practically nonirritating to the skin of rabbits.

004937

EPA: 68-01-6561
TASK: 123
September 3, 1985

DATA EVALUATION RECORD

RH-53,866

Primary Eye Irritation Study in Rabbits

STUDY IDENTIFICATION: Krzywicki, K. M., and Bonin, R. Primary eye irritation study in rabbits. (Unpublished study No. 84R134A prepared and submitted by Rohm and Haas, Spring House, PA; dated August 3, 1984.) Accession No. 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 9-3-85

1. CHEMICAL: RH-53,866; 2-butyl-2-(4-chlorophenyl)-1H-1,2,4-trazole-1-propanenitrile.

2. TEST MATERIAL: RH-53,866, technical grade, was an amber solid with 91.9 percent active ingredient.

3. STUDY/ACTION TYPE: Primary eye irritation study in rabbits.

4. STUDY IDENTIFICATION: Krzywicki, K. M., and Bonin, R. Primary eye irritation study in rabbits. (Unpublished study No. 84R134A prepared and submitted by Rohm and Haas, Spring House, PA; dated August 3, 1984.) Accession No. 072896.

5. REVIEWED BY:

William M. Butler, Jr., M.S.
Principal Reviewer
Dynamac Corporation

Signature: William M. Butler Jr.
Date: 8-30-85

Peggy Perreault, B.S.
Independent Reviewer
Dynamac Corporation

Signature: Peggy Perreault
Date: 8-30-85

6. APPROVED BY:

Robert Weir, Ph.D.
Acute Toxicology
Technical Quality Control
Dynamac Corporation

Signature: Robert Weir
Date: 8-30-85

Jane Harris, Ph.D.
EPA Reviewer and
Section Head

Signature: Jane E. Harris
Date: 8/31/85

7. SUMMARY:

Nine male New Zealand white rabbits were used in this study. The animals were obtained from Hazleton Dutchland, Denver, PA; the rabbits weighed between 2.0 to 3.5 kg. The rabbits were acclimated at least 6 days prior to dosing and were presented with rabbit chow and water ad libitum. One day prior to dosing the rabbits' eyes were grossly examined, then a drop of 2.0 percent sodium fluorescein was instilled into the eyes and the eyes were flushed with water. Only rabbits with undamaged eyes were used.

The test material was an amber solid, which was ground to a fine powder with a Bel-Art micromill. A dose of 100 mg of the finely ground powder was applied to the cornea of one eye of each of the nine rabbits. The eyelids were held open momentarily after dosing, released gently, and the animals were allowed to blink. The cornea and the surrounding areas were observed to be covered with the test substance; however, about 30 percent of the test substance was blinked off or fell from the treated eyes. Six of the rabbits' eyes were unwashed and were used to observe the irritation potential according to the Draize procedure. The remaining three rabbits' eyes were irrigated with water for approximately 60 seconds beginning 20 to 30 seconds after dosing. The eyelids and surrounding fur were blotted with a paper towel. Each treated eye was examined for irritation potential at 24, 48, and 72 hours and then again at 7, 14, and 21 days after dosing. The untreated eyes served as controls.

In each of the six unwashed rabbit eyes, three had test material around the conjunctivae at 24 and 48 hours and one at 72 hours. Three of the rabbits had the test material in the eyes only at the 24-hour observation period. The test material was observed around the treated eye of one of the rabbits that had been irrigated. Irritation to the cornea and conjunctiva was observed in six rabbits at 24 hours. The irritant effect on the cornea was reversed in two animals by 48 hours, three animals by 72 hours, and all six animals by day 7. The irritant effect to the conjunctiva was reversed in four rabbits by day 7 and in all six rabbits by day 14. Irritation to the iris was observed in three rabbits at 24 hours, but was reversed by 48 hours. Other evidences of irritation to the treated, unwashed eyes were the appearance of a hazy yellow area over the cornea and an uneven pitted area in the center of the cornea of one rabbit at 24 hours. Vascularization extended into the cornea of the eye of one rabbit, and a distinct 3-mm hazy yellow area was observed on the cornea of another rabbit following 2 percent fluorescein staining; both lasted throughout the 21-day observation period.

Based on duration of the ocular effects for greater than 21 days, the test substance, RH-53,866 is severely irritating to the eyes of rabbits. Irrigating the treated eyes of the rabbits, after exposure to the test material, prevented corneal effects, decreased conjunctival effects, and shortened the recovery period.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The design, conduct, and reporting of this study was generally acceptable. A quality assurance statement was not included in this report; however, the protocol indicated that the study should have been performed according to good laboratory practices.

9. CLASSIFICATION:

Core Classification: Core minimum.

Eye Irritation Results: The test material, RH-53,866, is a severe eye irritant in rabbits.

Toxicity Category: I.

004937

EPA: 68-02-4225
DYNAMAC No. 1-033-B[illegible],2
January 27, 1986

DATA EVALUATION RECORD

RH-53,866

Subchronic Oral Toxicity Study in Rats

STUDY IDENTIFICATION: O'Hara, G. P. and DiDonato, L. J. Three month dietary toxicity study in rats. (Unpublished study No. 83R-068 prepared by the Toxicology Department, Rohm and Haas Co., Spring House, PA, for Rohm and Haas Co., Philadelphia, PA; dated August 7, 1984.) Accession Nos. 072897-072898.

APPROVED BY:

I. Cecil Felkner, Ph.D.
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Date: 1-27-86

1. CHEMICAL: RH-53,866; RH-3866; alpha-butyl-alpha-4-chlorophenyl-1H-1,2,4-triazole-1-propanenitrile.

2. TEST MATERIAL: RH-53,866 (81.1% active ingredient) from lot No. LSPL00161E, TD No. 83-076, was described as a brown solid.

3. STUDY/ACTION TYPE: Subchronic oral toxicity study in rats.

4. STUDY IDENTIFICATION: O'Hara, G. P. and DiDonato, L. J. Three month dietary toxicity study in rats. (Unpublished study No. 83R-068 prepared by the Toxicology Department, Rohm and Haas Co., Spring House, PA, for Rohm and Haas Co., Philadelphia, PA; dated August 7, 1984.) Accession Nos. 072897-072898.

5. REVIEWED BY:

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7. CONCLUSIONS:

Although mixed function oxidase activity was increased at 300 ppm or greater RH-53,866 in the males and at 1,000 ppm or greater in the females, this is not considered an effect for the purposes of establishing a toxicity NOEL, but rather an indication of the power of accommodation of the liver. The LOEL is considered to be 3,000 ppm (150 mg/kg/day) on the basis of gross changes in the liver, increases in relative and absolute liver and relative kidney weights, and histopathologic changes in the liver and kidneys. Hence, the NOEL for systemic effects is considered to be 1000 ppm (equivalent to an actual intake of 50 mg/kg/day).

Core Classification: Core Minimum.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. The test material was liquefied in a water bath at 60°C, homogenized, dissolved in acetone, mixed with feed in a Hobart mixer, and added to the control diet. The diets were tested for homogeneity, and selected samples were evaluated by an unstated analytical procedure.

2. The test animals, COBS-CD(SD) BR strain rats (90 males and 90 females, 25-28 days old), were received from Charles River Breeding Laboratories, Kingston, NY, and a 4-week quarantine period was observed before the start of dosing. Animals were randomized, stratified by body weight, into dosage groups, each containing 10 males and 10 females, and individual animals were identified with a metal tag bearing a unique number. Animals were housed singly in stainless steel cages in an animal room that was environmentally controlled at 72°F, 40-60% humidity, and a 12-hour light cycle.

3. The diet was Purina Rodent Laboratory Chow (meal) No. 5001. Water and diets were available ad libitum. Groups of 10 rats/sex were fed control diets (designated Group 01) and test diets containing 5, 15, 50, 150, 500, 1,500, 5,000 or 15,000 ppm test material (respectively, Groups 02, 03, 04, 05, 06, 07, 08, and 09). During weeks 3 and 4 doses were increased to 7, 21, 70, 210, 700, 2,100, 7,000, or 21,000 ppm. During weeks 5-13, doses were increased to 10, 30, 100, 300, 1,000, 3,000, 10,000, or 30,000 ppm.

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[1] Only items appropriate to this DER have been included.

4. Animals were observed daily for signs of toxicity, ill health, morbidity, and mortality. Physical examinations were performed weekly. Body weights and feed consumption were measured weekly. Hematologic (7 tests) and clinical chemistry (14 tests) values were measured at 4 and 13 weeks of dosing on all surviving animals. Urinalysis was performed on all males and females of the control and 3,000- and 10,000-ppm dose groups after week 11. Ophthalmoscopic evaluation was conducted at initiation and termination.

5. At termination, survivors were killed and necropsies were performed. Organ weights were recorded for adrenals, brain, gonads, kidneys, liver, spleen, and thyroid/parathyroid (post-fixation). Sections of liver tissues from three rats/sex/dose were taken for determination of aminopyrine N-demethylase and benzphetamine N-demethylase activities in the groups receiving 0, 100, 300, 3,000 and 10,000 ppm RH-53,866. Approximately 35 tissues were taken for histopathologic examination, and these examinations were conducted on the control and 3,000- and 10,000-ppm groups; however, target organs were examined histopathologically at all concentration levels.

6. Normality of distribution of data and homogeneity of variance across groups were assessed by examination of the residual plots. Body weight and food consumption were evaluated by analysis of covariance using pretest values as the covariant.

   Group means were compared using a t-test. Analysis of variance was performed for some parameters on both sexes combined and also on the separate sexes for other parameters. Group means were compared using Duncan's multiple range test.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. Diet Analysis: Analysis of dietary samples for RH-53,866 revealed good approximation between the nominal and analyzed dietary concentrations. Dose levels ranged between 80 and 130% of intended concentrations, and the average concentration of test material was 103% of the nominal.

B. Survival and Clinical Observations: All rats treated with 30,000 ppm RH-53,866 for the last 8 weeks of this 13-week study (Group 09) died during the dosing period, the earliest deaths were after 17 and 18 days of dosing following dosage increase (i.e., during study week 8) in males and females, respectively. Signs of toxicity in those rats included a brown-stained, anogenital area, red or brown muzzle, scant feces, and emaciation. No dose-related deaths occurred at 10,000 ppm or lower; however, one male receiving 10,000 ppm was found dead on day 83. No dose-related signs of toxicity were observed at 10,000 ppm or lower doses.

C. Mean Body Weights and Food Consumption: The parameters were significantly decreased ($p < 0.05$) in both sexes at 30,000 and 10,000 ppm. At 3,000 ppm of RH-53,866, male body weights were decreased from weeks 6-12 without a corresponding decrease in food consumption. Food consumption was significantly decreased at 30,000 ppm of RH-53,866 for both males and females. At 10,000 ppm, food consumption was decreased for the males throughout the dosing period and was decreased for the females for the first 11 weeks of dosing. Table 1 presents selected body weight data.

D. Hematology: The hematologic effects in the 30,000 ppm group (measured at week 4 after dosage change) were considered compromised because of the debilitated condition of the animals. They included increased hematocrits (HCT), increased hemoglobin (HGB) and red blood cell counts (RBCs), decreased white blood counts (WBCs) and platelets, increased segmented neutrophils, and decreased lymphocytes and monocytes. In the 10,000-ppm group, effects included increased platelet counts and decreased mean corpuscular volume (MCV) and hemoglobin values [Table 2 (males) and Table 3 (females)]. The authors considered all of the effects related to the test material; however, the magnitude of the effects were slight and were not considered toxicologically significant.

E. Clinical Chemistry: The clinical chemistry findings are presented in Tables 4 and 5 for the males and females, respectively. Changes were noted at doses of 3,000 and 10,000 ppm; no compound-related effects were noted at 1,000 ppm or below. SGOT activity was significantly reduced in the males at 3,000 and 10,000 ppm. Reduced SGOT values have no biological meaning. SGPT was significantly increased in the males only at the 10,000-ppm dose for both intervals. The increased value at 100 ppm in the females is not meaningful and it does not follow a dose-effect relationship.

Cholesterol levels were generally increased in both sexes at 3,000 and 10,000 ppm. When the sexes were combined for analysis, alkaline phosphatase activity was significantly increased at 10,000 ppm; when the sexes were analyzed separately there was not a significant difference relative to the control level. BUN was elevated at 10,000 ppm in both sexes at both intervals. The serum phosphorus level was significantly decreased in the 10,000-ppm male group and significantly increased in the females; however, the study authors considered this to be of no toxicologic significance. Serum calcium was marginally increased in the 10,000-ppm males only at the 13-week interval. GGT activity was increased in both males and females of the 10,000-ppm group, but only at the 13-week interval. Total protein levels were increased in the 10,000-ppm dose for both sexes. Globulin levels were increased in both sexes at 10,000 ppm and in the males at 3,000 ppm. This was reflected in the A/G ratio, which was reduced in the 10,000-ppm female group when compared to the controls.

TABLE 1. Summary of Selected Mean Body Weights (g) and Food Consumption (g/rat/day) at Selected Intervals for Male and Female Rats Fed RH-53,866 in the Diet for 3 Months

Dose Levels (ppm)	Males			Females		
	Week			Week		
	0	6	13	0	6	13
	Body Weight					
0	294±14	437±23	517±28	184±10	248±18	285±25
100	286±12	423±28	504±45	181± 6	243± 9	282±18
300	296±18	437±27	522±42	182±12	247±20	285±25
3,000	292±16	408±25*	476±28	181±10	239±16	269±20
10,000	298±16	326±35*	365±54*	181±11	226±20*	244±29*
30,000	296±18	175±52*	--	182±12	127±19*	--
	Food Consumption					
0	27.1±1.3	27.0±1.3	25.2±1.0	18.2±1.2	19.8±1.8	17.2±2.0
100	25.9±2.3	26.2±2.0	25.0±3.4	17.8±1.1	20.1±1.3	18.9±1.6*
300	26.8±2.3	26.5±1.5	25.8±2.6	17.4±1.0	19.8±2.0	18.4±1.4*
3,000	26.7±1.3	25.8±2.2	24.9±2.1	17.8±1.2	19.0±1.5	18.4±1.4
10,000	27.7±2.1	21.9±3.4*	23.4±4.7	18.0±0.9	17.2±3.2*	17.7±2.8
30,000	27.3±2.1	14.6±3.8*	--	18.1±2.2	9.4±6.3*	--

*Significantly different from the control value ($p < 0.05$).

TABLE 2. Summary of Selected Mean Hematology Data at Selected Dose Levels Following Administration of RH-53,866 in the Diet of Male Rats for 3 Months

Test	Week	Dose Level		
		0 ppm	3,000 ppm	10,000 ppm
HCT (%)	46	61.8 ± 4.5	61.2 ± 2.2	58.5 ± 3.9
	13	59.0 ± 7.6	59.2 ± 4.1	57.6 ± 4.4
HGB (g/100 mL)	4	15.79 ± 0.90	15.58 ± 0.60	14.97 ± 0.96*
	13	15.81 ± 1.90	15.91 ± 0.73	15.40 ± 0.66
RBC (10^6/mm^3)	4	8.99 ± 0.72	9.12 ± 0.20	8.84 ± 0.67
	13	9.37 ± 1.33	9.69 ± 0.40	9.97 ± 0.41
Platelet (10^3/mm^3)	4	843 ± 124	929 ± 99	972 ± 131
	13	934 ± 209	906 ± 124	1095 ± 135*
MCV (m^3)	4	68.4 ± 2.1	67.1 ± 1.8	66.2 ± 1.9
	13	63.2 ± 3.8	60.9 ± 3.5	57.9 ± 3.9*
WBC (10^3/mm^3)	4	14.6 ± 3.5	16.7 ± 3.9	15.6 ± 3.4
	13	10.7 ± 2.3	10.3 ± 2.3	11.2 ± 3.9

*Significantly different from control value ($p < 0.05$).

TABLE 3. Summary of Selected Mean Hematology Data at Selected Dose Levels Following Administration of RH-53,866 in the Diet of Female Rats for 3 Months

Test	Week	Dose Level 0 ppm	3,000 ppm	10,000 ppm
HCT (%)	4	59.5 ± 2.9	59.8 ± 2.7	59.9 ± 2.9
	13	58.3 ± 3.0	56.5 ± 3.1	55.3 ± 2.6
HGB (g/100 mL)	4	15.4 ± 0.6	15.2 ± 0.5	15.0 ± 0.7
	13	15.7 ± 0.5	15.3 ± 0.7	14.8 ± 0.5*
RBC (10^6/mm^3)	4	8.48 ± 0.45	8.58 ± 0.42	8.75 ± 0.47
	13	8.71 ± 0.45	8.74 ± 0.42	9.31 ± 0.38
Platelet (10^3/mm^3)	4	884 ± 118	911 ± 139	1009 ± 89*
	13	936 ± 75	933 ± 114	1099 ± 130
MCV (m^3)	4	70.0 ± 0.9	69.7 ± 0.8	68.4 ± 1.4*
	13	66.8 ± 2.5	64.7 ± 4.3	59.6 ± 4.1*
WBC (10^3/mm^3)	4	11.7 ± 5.1	11.7 ± 3.1	14.9 ± 3.9
	13	6.9 ± 1.7	7.3 ± 3.0	7.8 ± 3.0

*Significantly different from control value ($p < 0.05$).

TABLE 4. Summary of Selected Mean Clinical Chemistry Data at Selected Dose Levels Following Administration of RH-53,866 in the Diet of Male Rats for 3 Months

Test	Week	Dose Levels 0 ppm	3,000 ppm	10,000 ppm
SGOT (U/L)	4	75.6 ± 19.7	63.0 ± 18.1	61.0 ± 20.7
	13	107.9 ± 21.1	83.0 ± 18.5*	84.1 ± 22.4*
SGPT (U/L)	4	22.7 ± 3.1	22.5 ± 4.6	29.2 ± 9.0*
	13	26.6 ± 2.6	25.1 ± 6.3	38.8 ± 10.0*
Cholesterol (mg/dL)	4	46.4 ± 11.3	59.5 ± 10.4	121.1 ± 14.8*
	13	58.9 ± 19.3	74.9 ± 11.6*	141.4 ± 24.0*
Creatinine (U/L)	4	72.3 ± 17.9	70.5 ± 22.8	68.7 ± 27.2
	13	53.1 ± 17.3	59.3 ± 15.6	66.4 ± 15.9
BUN (mg/dL)	4	14.4 ± 2.0	15.5 ± 2.2	18.6 ± 3.4*
	13	12.3 ± 1.9	13.1 ± 2.0	18.4 ± 2.0*
Phosphorus (mg/dL)	4	8.5 ± 0.7	7.9 ± 0.6	7.4 ± 0.8*
	13	6.7 ± 0.6	6.4 ± 0.6	6.1 ± 0.4*
CA^{++} (mg/dL)	4	11.23 ± 0.53	11.15 ± 0.71	11.28 ± 0.40
	4	10.55 ± 0.47	10.73 ± 0.63	11.27 ± 0.70*
GGT (U/L)	4	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 1.5
	13	0.0 ± 0.0	0.0 ± 0.0	8.9 ± 4.0*
Total Protein	4	6.16 ± 0.21	6.29 ± 0.28	6.84 ± 0.39*
	13	6.25 ± 0.37	6.32 ± 0.41	6.92 ± 0.6*
Globulin (g/dL)	4	2.72 ± 0.25	2.91 ± 0.30*	3.21 ± 0.33*
	13	2.75 ± 0.48	2.79 ± 0.45	3.14 ± 0.64
A/G Ratio	4	1.27 ± 0.14	1.18 ± 0.16	1.14 ± 0.14
	1	1.33 ± 0.37	1.30 ± 0.27	1.26 ± 0.32

*Significantly different from control value. ($p < 0.05$).

TABLE 5. Summary of Selected Mean Clinical Chemistry Data at Selected Dose Levels Following Administration of RH-53,866 in the Diet of Female Rats for 3 Months

Test	Week	DOSE LEVELS 0 ppm	3,000 ppm	10,000 ppm
SGOT (U/L)	4	75.1 ± 20.4	65.3 ± 12.8	55.7 ± 9.0
	13	87.9 ± 19.6	95.5 ± 23.8	77.1 ± 18.6
SGPT (U/L)	4	24.8 ± 4.5	18.7 ± 3.4	23.8 ± 6.9
	13	32.1 ± 6.3	25.1 ± 6.7	26.3 ± 5.4
Cholesterol (mg/dL)	4	52.4 ± 13.8	87.6 ± 20.2*	132.6 ± 15.8*
	13	64.8 ± 17.2	109.6 ± 11.3*	182.5 ± 25.4*
ALK Phos. (U/L)	4	54.7 ± 16.1	51.3 ± 16.5	55.9 ± 22.8
	13	44.2 ± 16.2	38.1 ± 16.1	58.2 ± 24.6
BUN (mg/dL)	4	15.7 ± 2.8	19.6 ± 2.0	21.5 ± 5.1*
	13	15.0 ± 2.3	17.6 ± 1.8	21.9 ± 2.7*
Phos (mg/dL)	4	7.7 ± 0.7	7.9 ± 0.7	7.6 ± 0.6
	13	5.5 ± 0.9	5.9 ± 0.8	6.5 ± 0.6*
Ca^{++} (mg/dL)	4	11.40 ± 0.32	11.40 ± 0.35	11.76 ± 0.35
	13	11.03 ± 0.31	10.77 ± 0.65	11.13 ± 0.49
GGT (U/L)	4	0.0 ± 0.0	1.8 ± 1.9	2.9 ± 1.4
	13	0.0 ± 0.0	0.4 ± 0.8	9.1 ± 4.3*
Total protein (g/dL)	4	6.52 ± 0.43	6.60 ± 0.68	7.04 ± 0.45*
	13	6.60 ± 0.64	6.85 ± 0.54	7.00 ± 0.64
Globulin (g/dL)	4	2.65 ± 0.33	2.98 ± 0.56	3.39 ± 0.41*
	13	2.47 ± 0.55	3.10 ± 0.59	3.35 ± 0.74*
A/G Ratio	4	1.48 ± 0.25	1.25 ± 0.24	1.10 ± 0.16*
	13	1.77 ± 0.55	1.27 ± 0.38	1.17 ± 0.44*

F. Urinalysis: No alteration in the urinalysis data could be associated with the administration of the test material.

G. Mixed Function Oxidase Studies: RH-53,866 produced increased hepatic mixed function oxidase (MFO) activity (Table 6) in the male rats at 300 ppm and greater and in females at doses of 1,000 ppm and greater. At 10,000 ppm, enzyme activities were increased as much as 6.5- and 8-fold in males and females, respectively. The authors considered these effects compound related, but not adverse toxicologic effects.

H. Ophthalmologic Evaluation: No ophthalmoscopic changes associated with the test material were observed. There were no dose-related patterns in the distribution of ocular abnormalities and no changes were considered related to dosing.

I. Gross Necropsy Findings: The 30,000-ppm dose group exhibited many compound-related effects in addition to those due to the extreme debilitation that occurred prior to death (all dead in 63 days). These effects included reddened lungs, small spleens, dark adrenal glands, red foci, or reddened mucosa of the stomach of both sexes. Small seminal vesicles were apparent in the males. The liver and kidney were dark.

The kidneys were darker than normal in the 10,000-ppm dose group. The liver architecture was prominent or accentuated in the males dosed with 1,000, 3,000, and 10,000 ppm and in the females dosed with 10,000 ppm. Darkened livers were seen in the 3,000-ppm males and in both sexes at doses of 10,000 and 30,000 ppm. Enlarged livers were observed in the males of the 3,000- and 10,000-ppm dose groups, but not in the females or in either sex at 30,000 ppm.

There were other miscellaneous observations occurring at low frequencies that were comparable in the control and dosed groups or which were unrelated to the compound dose.

J. Organ Weights: Significant ($p < 0.05$) changes in absolute and relative organ weights (see Table 7) when compared to control values were as follows:

1. Adrenal absolute weights were decreased in the 3,000- and 10,000-ppm male groups.

2. Brain absolute and relative weights were increased in the 10,000-ppm male group.

3. Relative gonad weights were increased in both males and females at 10,000 ppm.

4. The absolute heart weight for the 10,000-ppm male group was decreased whereas the relative weight was increased. In the females, there was no effect on absolute weights but the relative weights at doses of 3,000 and 10,000 ppm, respectively, were increased.

TABLE 6. Summary of Mean Microsomal Protein Concentration and Hepatic Mixed Function Oxidase (MFO) Activity in Male and Female Rats Fed RH-53,866 for 13 Weeks (Values were determined at termination.)

Dose Group (ppm)	Microsomal Protein (mg/g liver)	Nmol product formed/mg microsomal protein (Nmol/mg) AP[a]	(Nmol/mg) BP[b]	(Nmol/g liver) AP[a]	(Nmol/g liver) BP[b]
			MALES		
0	38.00 ±8.21	10.98 ±1.61	8.04 ±0.89	418.6 ±118.1	308.6 ± 89.2
100	49.04 ±6.34	12.01 ±3.05	7.62 ±1.75	587.4 ±152.6	371.9 ± 85.0
300	54.08*±1.60	14.35 ±2.29	9.86 ±1.51	778.5*±147.1	534.5*± 97.9
1,000	45.52 ±2.28	16.46*±1.47	15.81*±4.19	750.1*± 85.8	722.1*±203.4
3,000	62.64*±0.72	20.00*±2.47	25.09*±0.39	1253.8*±167.5	1570.9*± 10.0
10,000	72.96*±3.75	23.41*±1.80	27.31*±1.37	1712.4*±211.7	1994.8*±185.6
			FEMALES		
0	44.88 ±5.82	6.01 ±1.23	5.17 ±0.88	266.0 ± 31.5	230.1 ± 30.8
100	37.52 ±5.59	6.32 ±0.99	5.20 ±0.81	238.9 ± 63.2	196.2 ± 49.6
300	50.00 ±4.50	8.13 ±1.74	6.50 ±1.76	408.4 ±103.9	326.7 ± 97.0
1,000	47.52 ±4.08	10.75*±1.70	9.57*±1.64	513.7*±117.1	456.0 ±100.7
3,000	48.24 ±2.13	19.46*±1.44	19.94*±1.38	938.1*± 66.9	960.9*± 58.7
10,000	63.60*±8.52	24.48*±2.66	29.07*±5.34	1545.0*±111.0	1831.9*±263.0

[a]AP: aminopyrine N-demethylase.

[b]BP: benzphetamine N-demethylase.

*Significantly different from control value ($p < 0.05$).

TABLE 7. Selected Mean Absolute (Abs) and Relative (Rel) Organ Weights for Male and Female Rats Fed RH-53,866 in the Diet for 3 Months (Absolute values are in grams; relative values are organ weight x 1000/body weight.)

Dose Group (ppm)	Adrenal		Brain		Gonad		Heart		Kidneys		Liver		Spleen		Thyroid	
	(Abs)	(Rel)	(Abs)	(Rel)	(Abs)	(Rel)	(Abs)	(Rel)	(Abs)	(Rel)	(Abs)	(Rel)	(Abs)	(Rel)	(Abs)	(Rel)
MALES																
0	0.057	1.20	2.13	44.7	3.36	70.1	1.398	29.2	3.20	67.0	12.18	254	0.684	14.3	0.026	0.5
1,000	0.053	1.12	2.07	43.8	3.53	74.7	1.441	30.4	3.39	71.4	13.63	287	0.672	14.2	0.026	0.545
3,000	0.045*	1.00	2.10	47.4	3.67	82.9	1.375	31.0	3.51	79.0*	15.68*[a]	352*[a]	0.611	13.8	0.029	0.651*
10,000	0.044*	1.33	1.96*	59.5*	3.37	102.1*	1.168*	34.5*	3.10	91.9*	19.75*[a]	581*[a]	0.559*	16.6	0.025	0.742*
FEMALES																
0	0.067	2.55	1.92	73.2	0.116	4.43	0.818	31.2	1.90	72.1	6.44	246	0.385	14.7	0.021	0.792
1,000	0.067	2.64	1.89	74.2	0.115	4.46	0.815	31.9	1.87	73.1	7.06	276*[a]	0.428	16.7	0.020	0.765
3,000	0.068	2.77	1.88	76.1	0.129	5.22	0.843	33.9*	1.97	79.2*	8.17*[a]	329*[a]	0.437	17.6	0.018	0.746
10,000	0.057	2.57	1.86	84.1	0.138	6.09*	0.765	34.3*	1.81	80.8*	11.30*[a]	504*[a]	0.429	19.1*	0.021	0.9[illegible]*

*Significantly different from control value ($p < 0.05$).

[a]The reviewers calculated significant differences from the control for the liver weights only (absolute and relative) using Duncan's test; the indicated values were significant ($p < 0.01$).

5. Relative kidney weights were increased in both sexes at 3,000 and 10,000 ppm.

6. Absolute and relative liver weights were increased in both sexes at 3,000 and 10,000 ppm. In addition, the relative liver weight is also increased in the 1,000-ppm female group.

7. In the spleen, the absolute weight was decreased for the male whereas the relative spleen weight was increased for the female 10,000-ppm dose groups, respectively. Relative thyroid weight was increased in the male groups at 3,000 and 10,000 ppm; for the females in this dose group the relative thyroid weight was also increased. Many of the significant changes in organ-to-body weight ratios were the result of significantly ($p \leq 0.05$) decreased terminal body weight in males and females at 10,000 ppm and in males at 3,000 ppm.

K. Histopathologic Findings: Changes in the liver (Table 8) due to exposure at a dose of 10,000 ppm RH-53,866 consisted of centrolobular to panlobular hepatocellular hypertrophy, vacuolated hepatocytes, hepatocellular necrosis, and hepatocellular coagulation necrosis. Hypertrophy and hepatocellular necrosis occurred in both sexes at 3,000 ppm and above.

Pigmentation of the convoluted tubular epithelium of the kidney occurred at doses of 3,000 and 10,000 ppm in male animals only. This pigment was not positive for hemosiderin. In the male rats at 10,000 ppm as well as in the controls, hemosiderosis did occur in the red pulp of the spleen. Other compound-related histomorphologic changes included vacuolation of the adrenal cortices of both sexes at 3,000 and 10,000 ppm, an increase in small follicles of the thyroid in the 3,000- and 10,000-ppm males, and an increase in frequency of chronic alveolitis in the 10,000-ppm group of both sexes.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. "When fed to male and female rats for 3 months, RH-3866 has a NOEL of 100 ppm in the diet in males and 300 ppm in the diet in females. The only effect seen in males at 300 ppm was an increase in hepatic MFO activity. No adverse effects were seen at doses up to and including 1000 ppm."

B. The study protocol indicated that the study was to be conducted under good laboratory practices. A signed, but undated, quality assurance form was included in the report.

TABLE 8. Summary of Incidence of Liver Lesions in Male and Female Rats Fed RH 3866 for 90 Days

: (ppm)	Control		300		1000		3000		10,000	
	M	F	M	F	M	F	M	F	M	F
er of animals amined:	10	10	10	0	10	10	10	10	10	10
olobular hypertrophy h increased sinophilia	0	0	0	–	0	0	10	7	10	10
lated swollen patocytes	0	0	0	–	0	0	0	0	9	0
ocellular necrosis	0	0	0	–	0	0	1	3	1	1
metamorphosis	0	0	0	–	2	1	1	0	0	0
sis, coagulation, es	1	0	0	–	0	0	0	0	2	0

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The highest dose used in this study, 30,000 ppm, was clearly far in excess of the maximum tolerated level and produced total mortality (during study week 8). For this reason, little further attention will be given to this group. Although all doses referenced in all sections of this DER indicate an array of 0, 10, 30, 100, 300, 1,000, 3,000, or 10,000 ppm, it is important to remember that these doses were only given to the animal for weeks 5-13; for the first 2 weeks, the doses were only half of this level. Doses at weeks 3-4 were intermediate.

There was no effect on mortality and no compound-related clinical signs were observed at doses of 10,000 ppm or below. Body weight, however, was significantly depressed at 3,000 ppm and above in the male groups and at 10,000 ppm and above in the female groups. There was no correlation of growth to food consumption in the 3,000-ppm male groups, but there was body weight suppression that correlated with reduced food consumption in the 10,000- and 30,000-ppm groups of both sexes.

In the 10,000-ppm group, the decreased HGB and HCT counts together with decreased MCV values and increased RBC counts indicated that there was red cell destruction with compensatory red cell production. There was mild hemosiderosis in the spleens of the controls of both sexes and in 3,000 and 10,000 ppm groups. The hemosiderosis was more severe in the high dose males. Nevertheless, the red cell distruction was very slight and of little toxicologic significance with the exception of the high-dose males where hemosiderosis was evident.

The clinical chemical changes were many and varied, and many of these point to an adverse effect on the liver and kidney. Although some of the values were statistically significant, they are considered to be incidental or spontaneous in nature and are likely to be of little toxicological importance for the specific reasons that follow:

1. Reduced SGOT at 3,000 and 10,000 ppm; low values have no pathologic significance.

2. Phosphorus was reduced in the 10,000-ppm male group; however, phosphorus was increased in the 10,000-ppm female group.

3. Increased calcium levels in the 10,000-ppm groups of both sexes were of little toxicologic significance.

Although the authors concluded that only increased cholesterol and GGT were the only toxicologically adverse effects in the 10,000-ppm groups of both sexes, it is our assessment that increased SGPT at weeks 4 and 13 in the 10,000-ppm males, increased BUN at weeks 4 and 13 in the 10,000-ppm groups for both sexes, and increased total protein, globulin, and A/G ratios in these high-dose groups of both sexes were all induced by the test compound and are toxicologically meaningful. Clinical chemistry findings associated with RH-53,866 administration were not evident at doses below 10,000 ppm.

Mixed function oxidase activity was increased in males at doses of 300 ppm or greater and at doses of 1,000 ppm or greater in females. Gross necropsy findings were limited to the liver and were more pronounced in the males. They consisted of dark discolorations, enlargement, and/or prominent architecture at 1,000 ppm and above.

Increased absolute and relative liver weights were seen at 3,000 and 10,000 ppm in both sexes and increased relative liver weight occurred in the 1,000-ppm female group. Increased relative kidney weights were present in the 3,000 and 10,000 ppm doses in both sexes. Many of the other relative weight values were a result of decreased terminal body weights and are not toxicologically significant.

Histopathologic changes in the liver consisted of hepatocellular necrosis and hepatocellular hypertrophy at 3,000 ppm and above. At 3,000 or 10,000 ppm, pigmented convoluted tubules of the kidney were observed in males only. The adrenal cortex was vacuolated in both sexes at 3,000 and 10,000 ppm. An increase in small follicles was seen in the male thyroids at 3,000 ppm and above, and chronic alveolities occurred in the 10,000-ppm group of both sexes.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 340-346, and Appendix B, Protocol, CBI pp. 439-457.

APPENDIX A

Materials and Methods

MYCLOBUTANIL 128857

Page_____ is not included in this copy.

Pages 169 through 195 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

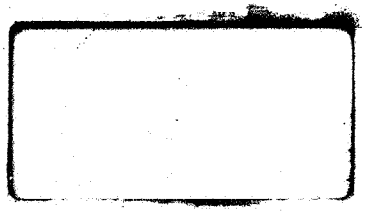
____ Information about a pending registration action.

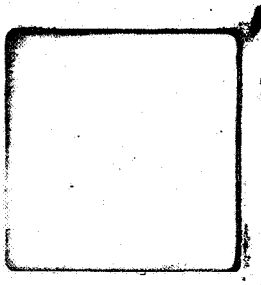
X FIFRA registration data.

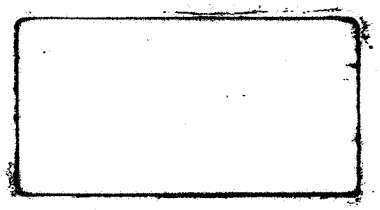
____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.







Addendum to Teratology Study in Rats Exposed to RH-53,866

The secondary reviewer, Dr. Jane E. Harris, disagrees with this interpretation that the increased incidences of 7th cervical ribs at the two highest doses are suggestive of a teratogenic potential of RH-53,866. Rather, the presence of such developmental variations as increased incidences of 7th cervical ribs and 14th rudimentary ribs in conjunction with the observed embryotoxicity, namely, an increased number of resorptions and decreased viability index suggest a fetotoxic effect. The absence of an increased incidence of soft tissue or skeletal malformations in rats exposed to RH-53,866 at doses up to 468.9 mg/kg support the conclusion that the increased incidences of 7th cervical and 14th rudimentary ribs at the two highest doses are developmental variations associated with fetotoxic effects.

EPA: 68-02-4225
DYNAMAC No. 33-01,2
December 31, 1985

DATA EVALUATION RECORD

RH-53,866

Teratogenicity Study in Rats

STUDY IDENTIFICATION: Costlow, R. D. and Kane, W. W. Teratology study with RH-53,866 in rats. (Unpublished study No. 83R-024 prepared and submitted by Rohm and Haas Company, Spring House, PA; dated June 22, 1984.) Accession No. 072901.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Departme .t Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 12-31-85

1. CHEMICAL: RH-53,866; alpha-butyl-alpha-4-chlorophenyl-1H-1,2,4-triazole-1-propanenitrile.

2. TEST MATERIAL: RH-53,866 (technical), lot No. LSPL 83-0017E, TD No. 83-087, was described as a viscous brown liquid consisting of 84.7% active ingredient.

3. STUDY/ACTION TYPE: Teratogenicity study in rats.

4. STUDY IDENTIFICATION: Costlow, R. D. and Kane, W. W. Teratology study with RH-53.866 in rats. (Unpublished study No. 83R-024 prepared and submitted by Rohm and Haas Company, Spring House, PA; dated June 22, 1984.) Accession No. 072901.

5. REVIEWED BY:

Patricia A. Turck, M.S.
Principal Reviewer
Dynamac Corporation

Signature: Patricia Turck
Date: 12/31/85

Robin B. Phipps, B.S.
Independent Reviewer
Dynamac Corporation

Signature: G Millicovsky FOR RBP
Date: 12/31/85

6. APPROVED BY:

Guillermo Millicovsky, Ph.D.
Teratogenic and Reproductive Effects
Technical Quality Control
Dynamac Corporation

Signature: G Millicovsky
Date: 12/31/85

Jane Harris, Ph.D.
EPA Reviewer/EPA Section Head

Signature: Jane E Harris
Date: 1/2/86

7. CONCLUSIONS:

A. The LOEL and NOEL for maternal toxicity in rats given oral doses of RH-53,866 from days 6 through 15 of gestation are 468.9 and 312.6 mg/kg/day respectively, based on slight decreases in body gain ~~weight~~ from GD 6-16 and clinical signs such as rough hair coat, salivation, alopecia, desquamation and red exudate around the mouth observed at 468.9 mg/kg/day after initiation of dosing.

The LOEL and NOEL for embryo/fetotoxicity are 93.8 and 31.3 mg/kg/day, respectively, based on slight increases in the mean number of resorptions noted at 93.8 and 312.6 mg/kg/day, marked increases in resorptions at 463.9 mg/kg/day, significant decreases in viability indices at 93.8 mg/kg/day and above, and significantly increased incidences of 14 rudimentary ribs and 7th cervical ribs at 312.6 and 468.9 mg/kg/day.

Since seventh cervical ribs are rare in rats, and since this anomaly is associated with clinical complications (nerve and blood vessel obstruction in the neck region), we assess that the dose-related increase of this finding suggests a teratogenic potential of RH-53,866.[2]

B. This study is classified as Core Minimum.

Item 8--see footnote 1.

9. BACKGROUND:

A range-finding teratogenicity study was conducted in rats to determine appropriate doses of RH-53,866 for a subsequent teratogenicity study. Daily oral dosages of 0, 31.6, 68.1, 100.0, 215.0, 464.4, or 700.0 mg/kg of test material in corn oil were administered to seven groups of eight mated Sprague-Dawley rats (Charles River) from gestational days (GD) 6 through 15.

All eight females in the 700-mg/kg dose group died before GD 20 (study termination), and two of eight females died in the 464.0-mg/kg dose group. Death was preceded by decreases in body weight, lethargy, ataxia, red exudate around the mouth, and rough hair coat. Maternal body weights were significantly decreased ($p < 0.05$) from GD 10 through study termination in the 464.4-mg/kg dose group when compared to controls. Findings at necropsy for the animals that died in the

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[1] Only items appropriate to this DER have been included.

2. See Addendum

in the 700-mg/kg dose group included reddened intestines, reddened and enlarged adrenals, reddened pancreas, hemorrhagic esophagus, and focal erosions in gastric mucosa, indicating that RH-53,866 at this dose level caused gastrointestinal irritation and stress. No compound-related maternal effects were noted at dosages below 464.4 mg/kg/day.

Pregnancy rates, number of corpora lutea and implantations, and implantation efficiency were comparable among control and test groups. The number of viable fetuses at 215.0 and 464.4 mg/kg and the viability index at 68.1 and 464.4 mg/kg of RH-53,866 were lower than controls. The number of resorptions was increased in all test groups compared to controls.

From results of this range-finding study, the authors concluded that the RH-53,866 was toxic to pregnant rats at 464.4 and 700.0 mg/kg.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. Test Material: Daily aliquots of technical RH-53,866, lot No. LSPL 83-0017E (84.5% active ingredient), were heated to 55°C, and 0.370, 1.1098, 3.6991, and 5.5487 g (adjusted for purity) of the test material were measured into volumetric flasks. The volumes were then adjusted to 100 mL by adding preheated (55°C) corn oil to obtain concentrations of 0, 31.26, 93.77, 312.58, and 468.87 mg/kg when administered in a dose volume of 10 mL/kg. The dose formulations were stirred continuously during dosing using a magnetic stir plate and were administered orally on GD 6 through 15; volumes were adjusted on weighing days for changes in body weight.

   Samples of the dose formulations were collected daily and analysis was conducted on the first, eighth, and last day of dosing. The methodology for analysis was not specified.

2. Animals and Test System: Virgin female Sprague-Dawley [Crl:CD-(SD)BR] rats approximately 63 days of age were received from Charles River Breeding Laboratories, Stone Ridge, NY, assigned temporary animal numbers, and acclimated for 20 days. During the acclimation period, animals within a body weight range of 2 standard deviations above or below the mean of the entire group of acclimating females were selected for the study. The animals were then randomized and assigned unique permanent numbers. At the end of the acclimation period, the females were placed in five groups of 25 animals each from lowest to highest consecutive number and mated to proven males of the same strain and source on a one-to-one

basis for up to 5 days. Eight "extra" females were mated and used as replacements for females on study that did not mate within 5 days. Vaginal smears were performed daily to confirm matings; the day on which sperm was noted was designated as GD 0.

The animals were observed daily for signs of mortality, toxicity, and general health, and body weights were measured on GD 0, 6, 10, 13, 16, 18, and 20.

Dams were sacrificed on GD 20 using carbon dioxide, and fetuses were delivered by Cesarean section. The uteri were examined for number and position of live and dead fetuses and early and late resorptions. The number of corpora lutea was recorded. Dams were then examined for visceral abnormalities; maternal tissues having gross lesions were fixed in 10% neutral-buffered formalin for possible histological examination. Fetuses were individually tagged according to protocol number, dam's identification number, and uterine position. Each live fetus was then weighed, sexed, and examined for external abnormalities. The authors did not specify the method of fetal sacrifice. Two-thirds of the fetuses from each litter were eviscerated, fixed in 95% ethyl alcohol, macerated in 2% aqueous potassium hydroxide, stained with Alizarin red S, and examined for skeletal abnormalities. The remaining one-third of the fetuses were fixed in Bouin's solution, sectioned, and examined for visceral abnormalities.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Test Material Analysis: The authors reported an error in the preparation of the dose formulations. The value for percent active ingredient used in the calculations was 81.1. However, the test material used in this study, lot No. LSPL 83-0017E, consisted of 84.5% active ingredient. The doses administered were therefore 4.19% higher than originally intended. The data were changed to reflect the adjustments; the actual doses were 31.3, 93.8, 312.6, and 468.9 mg/kg of RH-53,866.

Results of the chemical analysis indicated that the actual doses were between 88.3 and 102.7% of the target concentrations.

B. Maternal Effects: No mortalities occurred during the study. However, the following pharmacological observations were noted: rough hair coat, desquamation, salivation, alopecia, and urine stain. A summary of clinical observations is presented in Table 1. In addition, red exudate from the vagina and scant feces were exhibited by one and three animals, respectively, from the 468.9-mg/kg dose group. The authors considered the increased

TABLE 1. Effects of RH-53,866 on the Incidence of Selected Pharmacological Observations in Pregnant Rats

Observation	Incidence[a] at Dosage (mg/kg/day)				
	0	31.3	93.8	312.6	468.9
Number of mated females	25	25	25	25	25
Normal	22(525)	18(525)	17(525)	13(525)	3(525)
Alopecia	2 (2)	7 (42)	7 (63)	7 (67)	15(147)
Rough coat	0 (0)	0 (0)	1 (1)	4 (8)	8 (27)
Desquamation	0 (0)	0 (0)	0 (0)	1 (12)	5 (36)
Salivation	0 (0)	0 (0)	0 (0)	3 (3)	4 (4)
Red exudate from mouth	0 (0)	0 (0)	0 (0)	0 (0)	10 (22)
Urine stain	1 (2)	0 (0)	0 (0)	1 (1)	6 (17)

[a] Reported as the number of animals exhibiting the sign. Numbers in parentheses represent the total number of occurrences.

incidences of rough hair coat, desquamation, salivation, urine stain, and the presence of red exudate from the mouth to be compound-related effects. The authors discounted the alopecia, stating that hair loss frequently occurs in rats during gestation.

The authors reported slight decreases in maternal body weight gains (approximately 11%) between GD 6 through 16 of dosing at the 468.9-mg/kg level, but the only significant decrease occurred on GD 10 (Table 2). They further stated that a significant decrease in mean body weight occurred in the 31.3-mg/kg group on GD 13. However, statistical analysis of body weights by these reviewers revealed no significant differences in any dose group when compared to controls. Body weight gains were comparable between controls and the 93.8- and 312.6-mg/kg dose groups. No compound-related changes were observed at necropsy.

C. Embryonic/Fetal Effects: Pregnancy rates were comparable between control and test groups (Table 3). The authors stated that the numbers of corpora lutea and implantations for the test groups were within the range for historical controls, but values for the concurrent control group were slightly higher than those for historical controls; however, no statistical analyses were conducted on these parameters. Statistical analysis of implantation efficiencies revealed no differences between control and test groups. There were significant decreases ($p < 0.05$) in the number of viable fetuses per litter in all dose groups and the viability indices for the 93.8-, 312.6-, and 468.9-mg/kg dose groups when compared to controls (Table 3). No differences in fetal body weights or sex ratios occurred between control and test groups.

The effect of RH-53,866 on the incidences of variations and malformations is presented in Tables 4 and 5. Significant increases ($p < 0.05$) in the incidences of 7th cervical and 14th rudimentary ribs occurred in the 312.6- and 468.9-mg/kg dose groups when compared to controls. In addition, a significant dose-related trend of reduced ossification was reported, although no individual test group was significantly different from control values. No malformations were observed in the control or 312.6-mg/kg groups. Malformations reported in the 31.3-mg/kg group were microphthalmia in one fetus and atlo-occipital anomaly in a second fetus from a different litter. Several malformations occurred in the 93.8-mg/kg dose group; agnathia, anophthalmia, open eyelids, and misplaced pinna occurred in one fetus from one litter and an interventricular septal defect, retroesophageal aortic arch, and single atrium with a single atrioventricular valve occurred in one fetus from another litter. Craniorachischisis, a vertebral centra anomaly, and two incidences of hydrocephaly occurred in the 468.9-mg/kg dose group. Four fetuses from separate litters were affected, and the authors considered these findings random occurrences that were not compound related. While the number of malformed fetuses at 468.9 mg/kg was significantly higher ($p < 0.05$) than controls and a "marginally" significant dose-related trend was reported, the authors did not consider these findings toxicologically significant. No other compoundrelated changes occurred at any dose level.

TABLE 2. The Effect of RH-53,866 on the Mean Maternal Body Weights in Rats

Dosage (mg/kg/day)	Body Weight (g) at GD 0	6	10	13	16	20
0	261 ±15.9	292 ±16.9	296 ±17.4	310 ±17.8	330 ±23.5	399 ±31.0
31.3	259 ±14.2	281 ±18.1	288 ±16.7	307* ±21.1	323 ±20.6	391 ±24.0
93.8	255 ±14.7	280 ±15.6	286 ±17.9	299 ±17.4	320 ±20.6	386 ±23.8
312.6	259 ±15.8	283 ±16.3	290 ±20.6	304 ±21.1	325 ±22.1	390 ±26.3
468.9	262 ±13.9	289 ±15.3	288* ±16.8	305 ±15.8	323 ±18.7	392 ±28.8

	Body Weight Gain (g) at GD Interval 0-6	6-16	16-20	0-20
0	31	38	69	138
31.3	22	42	68	132
93.8	25	40	66	131
312.6	24	42	65	131
468.9	27	34	69	130

*Significantly different from controls at $p < 0.05$. Statistical analysis by these reviewers using ANOVA and Dunnett's t-test revealed no significant differences for any dosage group when compared to controls and no dose-related trend towards decreased body weight gain.

TABLE 3. The Effect of RH-53,866 on Reproductive Parameters in Rats

Parameters	Dosage (mg/kg/day) 0	31.3	93.8	312.6	468.9
No. females mated	25	25	25	25	25
No. females pregnant	22	24	21	23	23
Pregnancy rate (%)	88	96	84	92	92
Mean No. corpora lutea/litter	17.9	15.2	16.6	16.4	16.8
Mean No. implantations/litter	16.1	14.3	15.2	15.0	15.7
Implantation efficiency	0.91	0.94	0.93	0.91	0.94
Mean No. live fetuses/litter	15.3	13.5*	13.3*	13.2*	13.1*
Viability index[a]	0.95	0.95	0.88*	0.88*	0.83*
Mean No. resorptions	0.82	0.79	1.86	1.78	2.57
Mean fetal body wt. (g)	3.23	3.30	3.25	3.39	3.26
Fetal sex ratio[b]	0.99	1.14	1.07	1.43	0.94

[a] $\frac{\text{No. live fetuses}}{\text{No. implantation sites}}$

[b] $\frac{\text{No. male fetuses}}{\text{No. female fetuses}}$

*Significantly different from controls at $p < 0.05$.

TABLE 4. The Effect of RH-53,866 on Skeletal Findings in Rat Fetuses

	Number (% Incidence) at Dosage (mg/kg/day)				
	0	31.3	93.8	312.6	468.9
No. fetuses examined	223	213	185	200	201
No. litters examined	22	24	21	23	22
7th cervical rib					
Fetal	3(1.3)	9(0)	3(1.6)	17 (8.5)	45(22.4)
Litter	2(9.1)	0(0)	3(14.3)	10(43.5)*	14(63.6)*
14th rudimentary rib					
Fetal	1(0.4)	4(1.9)	1(0.5)	17 (8.5)	72(35.8)
Litter	1(4.5)	3(12.5)	1(4.8)	8(34.8)*	18(81.8)*
Reduced ossification of vertebrae					
Fetal	3(1.3)	1(0.5)	6 (3.2)	5 (2.5)	6 (3.0)
Litter	3(9.1)	1(4.2)	5(23.8)	4(17.4)	5(22.7)
Sternebrae not ossified[a]					
Fetal	8 (3.6)	2(0.9)	3 (1.6)	3(1.5)	8 (4.0)
Litter	5(22.7)	2(8.3)	3(14.3)	2(8.7)	7(31.8)
Sternum not ossified					
Fetal	1(0.4)	0(0)	0(0)	2(1.0)	0(0)
Litter	1(4.5)	0(0)	0(0)	2(8.7)	0(0)

*Significantly different from controls at $p < 0.05$.

[a]Sternebrae other than 5/6.

7. SUMMARY:

Groups of 10 male and 10 female CRCD albino rats (Charles River, Kingston, NY), weighing between 165 and 179 g, were administered by gavage single doses of 0, 0.8, 1.3, 2.0, or 3.2 g/kg of RH-53,866 2EC diluted with distilled water at a volume of 10 mL/kg. The rats were observed for 14 days post-dosing. All female rats and eight male rats given 3.2 g/kg died by day 3 of the observation period. Seven male rats and eight females given 2.0 g/kg and three males and six females given 1.3 g/kg died by day 2 of the observation period. One female rat given 0.8 g/kg died on day 5 and all of the male and female control rats survived through day 14. Pharmacotoxic signs noted included passiveness, prostration, ataxia, lacrimation, abdominal breathing, red- or tan-stained muzzle, and brown- or yellow-stained anogenital area. At necropsy, animals that died during the study had reddened lungs (females only), white or clear fluid-filled stomach, and white fluid-filled intestines; animals sacrificed at the end of the observation period exhibited no gross changes. Based on these data, the oral LD_{50} for RH-53,633 2EC in male rats is 1.80 (1.39-2.40) g/kg and 1.28 (0.98-1.60) g/kg for female rats, which corresponds to Toxicity Category III.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The data and conclusions appeared to be valid; however, a quality assurance statement was not presented. A statement that good laboratory practices were to be followed was included, but it was not signed and dated.

9. CLASSIFICATION:

Core Classification: Core Minimum.

Toxicity Category: III.

Acute Oral LD_{50}: Male rats--1.80 (1.309-2.40) g/kg.
Female rats--1.28 (0.98-1.60) g/kg.

212

004937

EPA: 68-01-6561
TASK: 123
September 11, 1985

DATA EVALUATION RECORD

RH-53,866

Primary Eye Irritation Study in Rabbits

STUDY IDENTIFICATION: Krzywicki, K. M., and Watts, M. H., Jr. Acute eye irritation, definitive, rabbits. (Unpublished study No. 84R-077A prepared and submitted by Rohm and Haas Co., Spring House, PA; dated July 16, 1984.) Accession No. 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 9-11-85

1. CHEMICAL: RH-53,866 2EC.

2. TEST MATERIAL: RH-53,866 2EC (emulsifiable concentrate formulation), material key 892334-7, TD No. 84-027, lot No. EG-0807-1, was described as a liquid containing 28.5 percent RH-53,866 Tech (24.0 percent active ingredient).

3. STUDY/ACTION TYPE: Primary eye irritation study in rabbits.

4. STUDY IDENTIFICATION: Krzywicki, K. M., and Watts, M. H., Jr. Acute eye irritation, definitive, rabbits. (Unpublished study No. 84R-077A by and for Rohm and Haas Co., Spring House, PA; dated July 16, 1984.) Accession No. 072896.

5. REVIEWED BY:

Peggy Perreault, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Peggy Perreault
Date: 9-10-85

Sharon Ambrose, B.S.
Independent Reviewer
Dynamac Corporation

Signature: Sharon Ambrose
Date: 9-10-85

6. APPROVED BY:

Finis Cavender, Ph.D.
Acute Toxicity
Technical Quality Control
Dynamac Corporation

Signature: Finis Cavender
Date: 9/10/85

Jane Harris, Ph.D.
EPA Reviewer and Section Head

Signature: Jane C Harris
Date: 9/11/85

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7. SUMMARY:

A. One eye of each of nine New Zealand white rabbits (Hazleton Dutchland, Denver, PA) with no ocular abnormalities was treated with 0.1 mL of RH-53,866 2EC. The untreated eye of each animal served as the control. The treated eyes of three rabbits were flushed with water for 60 seconds beginning 20 to 30 seconds after treatment whereas the treated eyes of the other six rabbits remained unwashed. Ocular reactions were scored according to Draize's Procedure 24, 48, and 72 hours and 7, 14, and 21 days after treatment. Irritation of the cornea, iris, and conjunctivae was observed at 24 hours, but appeared to decrease at 48 hours. Corneal and conjunctival effects increased at 72 hours through day 7, then decreased again, but persisted through day 21 in both unwashed and washed eyes. Effects on the iris steadily decreased after 24 hours and disappeared by day 14 in unwashed eyes and by day 7 in washed eyes. Other effects noted included vocalization in one animal after application of the test substance, blood vessels and/or circumcorneal blood vessels extending onto the cornea of all animals, hair loss around the treated eye of two animals, and a red discharge around one washed eye. Based on these data and the presence of ocular effects 21 days after treatment, the test substance, RH-53,866 2EC, is considered to be severely irritating to the eyes of rabbits, corresponding to Toxicity Category I.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The data appeared to be valid; however, eye irritation response data was not included for an observation period 4 hours after treatment as specified in the protocol (81P-60). The addendum to the protocol pertains to the dermal LD_{50} study for RH-53,866 and should not be included in this report. A quality assurance statement was not presented; however, a statement ensuring that good laboratory practices were to be followed was included, but was not signed nor dated.

9. CLASSIFICATION:

Core Classification: Core Minimum.

Toxicity Category: I.

Eye Irritant: Severe.

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004937

EPA: 68-01-6561
TASK: 123A-15
September 24, 1985

DATA EVALUATION RECORD

RH-53,866

Acute Inhalation Toxicity Study in Rats

STUDY IDENTIFICATION: Hagan, J. V., and Baldwin, R. C. Acute inhalation toxicity study in rats. (Unpublished study No. 84R-048 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated June 27, 1984.) Accession no. 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I Cecil Felkner

Date: 9-24-85

TABLE 5. The Effect of RH-53,866 on the Incidences of Malformations in Rat Fetuses

004937

	Number (% Incidence) at Dosage (mg/kg/day)				
	0	31.3	93.8	312.6	468.9
No. fetuses examined	223	213	185	200	201
No. litters examined	22	24	21	23	22
External Malformations:					
Craniorachischisis					
Fetal	0(0)	0(0)	0(0)	0(0)	1(0.5)
Litter	0(0)	0(0)	0(0)	0(0)	1(4.5)[c]
Agnathia					
Fetal	0(0)	0(0)	1(0.5)[a]	0(0)	0(0)
Litter	0(0)	0(0)	1(4.8)	0(0)	0(0)
Misplaced pinna					
Fetal	0(0)	0(0)	1(0.5)[a]	0(0)	0(0)
Litter	0(0)	0(0)	1(4.8)	0(0)	0(0)
Skeletal Malformations:					
Atlo-occipital anomaly					
Fetal	0(0)	1(0.5)	0(0)	0(0)	0(0)
Litter	0(0)	1(4.2)	0(0)	0(0)	0(0)
Vertebral centra anomaly					
Fetal	0(0)	0(0)	0(0)	0(0)	1(0.5)[d]
Litter	0(0)	0(0)	0(0)	0(0)	1(4.5)
Soft Tissue Malformations:					
Hydrocephaly					
Fetal	0(0)	0(0)	0(0)	0(0)	2(2.0)
Litter	0(0)	0(0)	0(0)	0(0)	2(9.1)
Open eyelids					
Fetal	0(0)	0(0)	1(0.5)[a]	0(0)	0(0)
Litter	0(0)	0(0)	1(4.8)[a]	0(0)	0(0)
Interventricular septal defect					
Fetal	0(0)	0(0)	1(0.5)[a]	0(0)	0(0)
Litter	0(0)	0(0)	1(4.8)	0(0)	0(0)
Atrium with single atrioventricular valve					
Fetal	0(0)	0(0)	1(0.5)[b]	0(0)	0(0)
Litter	0(0)	0(0)	1(4.8)	0(0)	0(0)
Retroesophageal aortic arch					
Fetal	0(0)	0(0)	1(0.5)[b]	0(0)	0(0)
Litter	0(0)	0(0)	1(4.8)	0(0)	0(0)

[a,b,c,d] The same superscript indicates the same fetus.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that the LOEL and NOEL for maternal toxicity in rats were 312.6 and 93.8 mg/kg of RH-53,866, respectively, based on clinical signs such as rough hair coat, urine stain, and salivation that developed at 312.6 and 468.9 mg/kg after initiation of dosing.

The authors stated that significant decreases in the numbers of live fetuses between control and test groups were not toxicologically significant because the mean litter size for the controls was greater than the range seen in historical control data whereas the values for the test groups were within the historical range. However, the authors considered the statistically significant differences in the viability index at the 93.8-, 312.6-, and 468.9-mg/kg doses to be compound-related occurrences. The authors concluded that the LOEL and NOEL for embryotoxicity in rats were 93.8 and 31.3 mg/kg of RH-53,866, respectively, based on significant decreases in the viability index and increases (not statistically analyzed) in the number of resorptions at 93.8, 312.6, and 468.9 mg/kg of RH-53,866. The LOEL and NOEL for fetotoxicity in rats were assessed to be 312.6 and 93.8 mg/kg, respectively, based on increases in developmental variations such as the 14th rudimentary and 7th cervical ribs at the 312.6- and 468.9-mg/kg dose levels and a significant dose-related trend of reduced ossification. According to the authors, teratogenic effects of RH-53,866 were not evident in this study. The significant increase in malformations reported in the 468.9-mg/kg dose group was not considered a teratogenic response because only four fetuses from separate litters were affected and the types of malformations that occurred were not related.

B. A signed quality assurance statement was present but not dated.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. 1. Maternal Effects: After initiation of dosing, clinical signs such as rough hair coat, salivation, alopecia, desquamation, and urine stains on a urogenital area were observed in the 312.6- and 468.9-mg/kg dose groups. Incidences of red exudate around the mouth and vagina were also observed in the high-dose group after initiation of dose administration suggesting minimal toxicity. In addition, small decrease in body weight gain was noted between 6 through 16 at the 468.9 mg/kg when compared to controls. Therefore, it appears that a more appropriate LOEL for maternal toxicity is 468.9 mg/kg with a NOEL of 312.6 mg/kg.

2. Embryonic/Fetal Effects: Pregnancy rates, number of corpora lutea, implantations, and implantation efficiency were comparable among control and test groups. The number of resorptions per litter was increased at the 93.8-, 312.6-, and 468.9-mg/kg doses; most resorptions occurred early in gestation, indicating an embryolethal effect. The number of viable fetuses per litter and the viability indices were significantly reduced ($p < 0.05$) at the 31.3-, 93.8-, 312.6-, and 468.9-mg/kg doses and the 93.8-, 312.6-, and 468.9-mg/kg doses, respectively, when compared to controls. We consider the increased incidence of resorptions and decreased litter viability evidence of embryonic/fetal lethality. Fetal body weights and sex ratios were comparable among control and test groups. The incidences of 7th cervical and 14th rudimentary rib were significantly increased ($p < 0.05$) at 312.6 and 468.9 mg/kg. Although severe malformations such as agnathia, craniorachischisis, and hydrocephaly appeared only in the dose groups and there appeared to be a significant increase in the incidence in malformations in the 468.9-mg/kg dose group when compared to controls, these occurred in very low incidences and not in a dose-related pattern. Therefore, we were unable to make a definitive assessment of the biological significance of these findings.

8. The following items are differences between the reviewers and study authors' interpretation and conclusions:

1. The study authors stated that since alopecia was generally observed in rats during gestation, the increased incidences of hair loss in the test groups were not toxicologically significant. However, the occurrence of alopecia at the 312.6- and 468.9-mg/kg doses was accompanied by desquamation. That is, desquamation occurred only in areas where hair loss was evident; this is not a normal occurrence in rats during gestation. Also, the incidence of alopecia was 2, 7, 7, 7, and 15 for the 0-, 363-, 93.8-, 312.6-, and 468.9-mg/kg groups, respectively, suggesting a compound-related increase; therefore, we assess that the alopecia observed is compound related.

2. We disagree with the study authors' conclusion that decreases in the number of viable fetuses per litter were artifactual and not toxicologically significant. Because the number of corpora lutea, implantations, and implantation efficiency were comparable between the control and test groups, we conclude that the smaller litter sizes in the test groups were due to compound administration. Furthermore, we do not think that comparison of data from the test groups to historical control data instead of to concurrent control data is conclusive.

3. Although the presence of 7th cervical ribs was considered by the authors to be indicative of fetotoxicity, we consider this finding as a permanent anatomical abnormality that is often associated with adverse physiological complications (nerve and blood vessel obstruction); in addition, this anomaly is rare in rats. Therefore, we assess that the dose-related increase of 7th cervical ribs suggests a teratogenic potential of the test material.*

C. The following deficiencies in the conduct and reporting of this study were noted:

1. The method of fetal sacrifice (if any) was not reported. Therefore, we were unable to determine if the method used was appropriate for a teratology study.

2. Individual fetal and litter body weights were not reported. Therefore, we were unable to independently verify data in the summary tables.

3. The method for confirming pregnancy status (when no visible implantation sites were observed) was not reported. We were, therefore, unable to assess if the method was appropriate for a teratology study.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Study Protocol, CBI pp. 94-105.

* See Addendum

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

004937

EPA: 68-01-6561
TASK: 123
September 11, 1985

DATA EVALUATION RECORD

RH-53,866

Acute Oral Toxicity Study in Rats

STUDY IDENTIFICATION: Watts, M. H., Jr., and Krzywicki, K. M. Acute oral LD_{50}, definitive, rats. (Unpublished study Nos. 84R-077A and 84R-077B prepared and submitted by Rohm and Haas Co., Spring House, PA; dated July 16, 1984.) Accession No. ~~072986~~ 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 9-11-85

1. CHEMICAL: RH-53,866 2EC.

2. TEST MATERIAL: RH-53,866 2 EC (emulsifiable concentrate formulation), material key 892334-7, TD No. 84-027, lot No. EG-0807-1, was described as a brown liquid containing 28.5 percent RH-53,866 Tech (24.0 percent active ingredient).

3. STUDY/ACTION TYPE: Acute oral toxicity study in rats.

4. STUDY IDENTIFICATION: Watts, M. H., Jr., and Krzywicki, K. M. Acute oral LD_{50}, definitive, rats. (Unpublished study Nos. 84R-077A and 84R-077B prepared and submitted by Rohm and Haas Co., Spring House, PA; dated July 16, 1984.) Accession No. ~~072986~~ 072896.

5. REVIEWED BY:

Peggy Perreault, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Peggy Perreault
Date: 9-10-85

Sharon M. Ambrose, B.S.
Independent Reviewer
Dynamac Corporation

Signature: Sharon M Ambrose
Date: 9-10-85

6. APPROVED BY:

Finis Cavender, Ph.D.
Acute Toxicology
Technical Quality Control
Dynamac Corporation

Signature: Finis Cavender
Date: 9/10/85

Jane E. Harris, Ph.D.
EPA Reviewer and
Section Head

Signature: Jane E Harris
Date: 9/11/85

004937

EPA: 68-02-4225
TASK: 033-A16
October 31, 1985

DATA EVALUATION RECORD

RH-53,866

Dermal Sensitization Study in Guinea Pigs

STUDY IDENTIFICATION: Murphy, M. E., and Chan, P. K. Delayed contact hypersensitivity study in guinea pigs. (Unpublished study No. 84R-084 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated July 30, 1984.) Accession No. 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 10-31-85

1. CHEMICAL: RH-53,866 2EC; α-butyl-α-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile.

2. TEST MATERIAL: RH-53,866 2EC, from lot No. EG-0807-1, sample No. 84-027, was described as a liquid containing 28.5 percent RH-53,866 technical, [redacted] The solvent/emulsifier of RH-53,866 2EC was described as an amber liquid containing [redacted]

3. STUDY/ACTION TYPE: Dermal sensitization study in guinea pigs.

4. STUDY IDENTIFICATION: Murphy, M. E., and Chan, P. K. Delayed contact hypersensitivity study in guinea pigs. (Unpublished study No. 84R-084 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated July 30, 1984.) Accession No. 072896.

5. REVIEWED BY:

Peggy Perreault, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Peggy Perreault
Date: 10-31-85

Patricia Turck, M.S.
Independent Reviewer
Dynamac Corporation

Signature: Patricia Turck
Date: 10/31/85

6. APPROVED BY:

Finis Cavender, Ph.D.
Acute Toxicity
Technical Quality Control
Dynamac Corporation

Signature: Finis Cavender
Date: [illegible]

Jane Harris, Ph.D.
EPA Reviewer and Section Head

Signature: Jane C. Harris
Date: 11/1/85

7. SUMMARY:

Fifteen male and fifteen female young adult Hartley guinea pigs (Charles River Kingston Breeding Labs, Stoneridge, NY) weighing between 364 and 549 g were used for the delayed contact hypersensitivity study. The guinea pigs were randomly assigned (computergenerated) to a naive control group (5 males and 5 females) or an induction group (10 males and 10 females).

One day prior to application of the test substance (both induction and challenge applications) the hair was clipped from the back of each guinea pig. The guinea pigs were placed in restrainers; the test substance was pipetted onto patches which were applied to the clipped area on each guinea pig and occluded with rubber dental dams. After a 6-hour exposure period, the animals were removed from the restrainers, the patches were removed, and the application sites were washed and dried. The clipped area on each guinea pig was divided into 6 different application sites. Site one, located over the left shoulder, was used for the induction phase. Sites 4 and 5, located over the right shoulder and the right mid-back area, respectively, were used for the challenge phase. The remaining 3 sites were not used. Guinea pigs in the control group were clipped but not dosed during the induction phase. A modified Buehler's method was used for dosing; ten induction doses (0.4 ml) of 100 percent RH-53,866 2EC were applied to the backs of each of the 20 induction group guinea pigs on consecutive Mondays, Wednesdays, and Fridays for six hours each day over a 3.5 week period. Two weeks after the last induction treatment, the 20 induction group animals and the control guinea pigs were each challenged with 0.4 ml of 12.5 percent of RH-53,866 2EC in distilled water (the highest non-irritating concentration) and 0.4 ml of 8.94 percent solvent/emulsifiers of RH-53,866 2EC in water. The concentrations of solvent/emulsifiers in both suspensions were equal.

Twenty-four hours after the challenge application, the back of each guinea pig was depilated with Neet® lotion hair remover. Erythema reactions were scored 2-5 hours after depilation and again at 48 hours after the challenge exposure. The results were evaluated by comparing the incidence of erythema reactions in the induction group to the incidence of reactions in the non-induced control group. No erythema of grade 1 (slight confluent or moderate patchy erythema) or greater was observed in either group (control or induction) after being challenged with RH-53,866 2EC at 12.5 percent in distilled water; the mean erythema scores for the induction group were 0.20 and 0.25 at 24 and 48 hours, respectively, whereas the score for the control group at both 24 and 48 hours was 0.05. After being challenged with the solvent/emulsifiers at 8.94 percent in distilled water, a grade 1 erythema was observed after 24 hours in one animal of each group (control and induction); the mean erythema score for the induction groups at 24 and 48 hours was 0.18, while the scores for the control group were 0.3 at 24 hours and 0.35 at 48 hours. Based on these data, the test substance, RH-53,866 2EC, did not produce delayed contact hypersensitivity (dermal sensitization) in guinea pigs.

8. REVIEWERS COMMENTS AND QUALITY ASSURANCE MEASURES:

The study design was adequate and the data was valid. A signed quality assurance statement was presented.

9. CLASSIFICATION:

Core Classification: Core Guideline.

Did not produce delayed contact hypersensitivity in guinea pigs.

004937

EPA: 68-01-6561
TASK: 123
September 24, 1985

DATA EVALUATION RECORD

RH-53,866

Primary Eye Irritation Study in Rabbits

STUDY IDENTIFICATION: Krzywicki, K. M. and Morrison, R. D. Acute eye irritation, definitive, rabbits. (Unpublished study No. 84R-082A prepared and submitted by Rohm and Haas Co., Spring House, PA; dated July 16, 1984.) Accession No. 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: Ira Cecil Felkner

Date: 9-24-85

1. CHEMICAL: RH-53,866 40WP.

2. TEST MATERIAL: RH-53,866 40WP from lot No. EG-0809-1, TD No. 84-039, was described as a white powder containing 39.5 percent active ingredient.

3. STUDY/ACTION TYPE: Primary eye irritation study in rabbits.

4. STUDY IDENTIFICATION: Krzywicki, K. M. and Morrison, R. D. Acute eye irritation, definitive, rabbits. (Unpublished study No. 84R-082A prepared and submitted by Rohm and Haas Co., Spring House, PA; dated July 16, 1984.) Accession No. 072896.

5. REVIEWED BY:

Peggy Perreault, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Peggy Perreault
Date: 9-24-85

Patricia Turck, M.S.
Independent Reviewer
Dynamac Corporation

Signature: James R. Plautz for PA
Date: 9-24-85

6. APPROVED BY:

Robert J. Weir, Ph.D.
Acute Toxicity
Technical Quality Control
Dynamac Corporation

Signature: Robert J. Weir
Date: 9/24/85

Jane Harris, Ph.D.
EPA Reviewer and Section Head

Signature: Jane E Harris
Date: 9/27/85

7. SUMMARY:

One eye of each of nine male New Zealand white rabbits (Hazleton Dutchland, Denver, PA) with no ocular abnormalities was treated with 0.1 g of RH-53,866 40WP. The untreated eye of each animal served as the control. The test substance was applied to the corneal surface of the eye. The eye was held open momentarily after application and then released gently. Approximately 40 percent of the test substance was blinked or fell from the eye; however, the cornea and surrounding area were observed to be covered with the test substance. The treated eyes of three rabbits were flushed with water for 60 seconds beginning 20 to 30 seconds after treatment, whereas the treated eyes of the other six rabbits remained unwashed. Ocular reactions such as irritation of the cornea, iris, and conjunctiva were scored according to the system of Draize 24, 48, and 72 hours and 7 days after treatment. Irritation of the iris was observed at 24 hours in the unwashed eye of one animal and disappeared by 48 hours. Corneal and conjunctival effects, which were also observed at 24 hours, disappeared by 72 hours. Another effect noted was vocalization in two animals after application of the test substance. One animal was found dead at 24 hours; however, based on the oral and dermal toxicity of the test substance, the authors concluded that this death was probably not treatment related. Based on the reversal of ocular effects within 7 days but not 24 hours, the test substance, RH-53,866 40WP, is moderately irritating to the eyes of rabbits, corresponding to Toxicity Category III.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The data appeared to be valid; however, a quality assurance statement was not presented and the protocol (81P-60) was not followed. Eye irritation response data was not reported for 4 hours after treatment as specified in the protocol nor was a protocol amendment presented. According to EPA Guidelines, the dosing technique that was used was incorrect; the eye was held open momentarily after application of the test substance and 40 percent of the test material fell out. The eye should have been held shut for 1 second to prevent loss of the test material. Had the correct dosing technique been used, the resulting eye irritation would probably have been more severe. Based on the inadequate study design, this study provides supplementary data.

9. CLASSIFICATION:

Core Classification: Supplementary.

Toxicity Category: III.

Eye Irritation: Moderately irritating to the eyes of rabbits.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

004537

EPA: 68-02-4225
DYNAMAC No. 1-33A-14R
February 3, 1986

SPECIAL REPORT--RH-53866

Evaluation of the Rohm and Haas Company's
Response (Chan, P. K.; TD 86M-60; pp. 2,3) to the DER--
Primary Eye Irritation Study in Rabbits
(Study No. 84R-082A dated July 16, 1984, Accession No. 072896)
Prepared by Dynamac Corporation (No. 1-33A-14)

REVIEWED BY:

Robert J. Weir, Ph.D.
Senior Reviewer
Dynamac Corporation

Signature: [signature]
Date: 2-3-86

APPROVED BY:

I. C. Felkner, Ph.D.
Department Director
Dynamac Corporation

Signature: [signature]
Date: 2-3-86

Jane Harris, .D.
EPA Reviewer and
Section Head

Signature: _____
Date: _____

7. SUMMARY:

The reviewers are aware of the work of Beckley (1965) and Griffith (1980) as well as NAS publication No. 1138 (1977). The latter was prepared primarily at the request of the Consumer Product Safety Commission (CPSC).

The reviewers believe that the method used by Rohm and Haas, which is in agreement with the above citations, is inappropriate because:

1. It is an ineffective method by which to control dose, as exemplified by the loss of an estimated 40% of the test material in the study reviewed.

2. It does not represent a method supported by extensive historical information that allows one to extrapolate animal studies to the potential effect in man. Information in the bulk of the literature on primary eye studies indicates that the application is made by placing the test substance in the sac formed between the lid and the conjunctiva and holding the lid closed for 1 second.

EPA guidelines recommend the application to the conjunctival sac and holding the lid closed for 1 second. Even the CPSC does not recommend method of the application provided in the NAS publication, but rather that method given in the EPA guidelines.

The potential of trapping material in the sac and the possibility of mechanical damage to the eye would also be a potential in human exposure, and as such is not a source for considerable error or inaccuracy.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

004937

EPA: 68-02-4225
TASK: 033-A17
October 31, 1985

DATA EVALUATION RECORD

RH-53,866

Acute Inhalation Toxicity Study in Rats

STUDY IDENTIFICATION: Hagan, J. V., and Baldwin, R. C. Acute dust inhalation toxicity study in rats. (Unpublished Study No. 84R-047 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated June 27, 1984.) Accession No. 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 10-31-85

1. CHEMICAL: RH-53,866 40WP (α-butyl-α-(4-chlorophenyl)-1H-1,2,4-triazole-1-propanenitrile).

2. TEST MATERIAL: RH-53,866 40WP, sample No. 84-39, lot No. EG-0809-1, was described as a buff powder containing 44.0 percent RH-53,866 technical.

3. STUDY/ACTION TYPE: Acute inhalation toxicity study in rats.

4. STUDY IDENTIFICATION: Hagan, J.V., and Baldwin, R.C. Acute dust inhalation toxicity study in rats. (Unpublished Study No. 84R-047 prepared and submitted by Rohm and Haas Co., Spring House, PA; dated June 27, 1984.) Accession No. 072896.

5. REVIEWED BY:

Peggy Perreault, B.S.
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Signature: Peggy Perreault
Date: 10-31-85

Patricia Turck, M.S.
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Date: 10/31/85

6. APPROVED BY:

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Acute Toxicity
Technical Quality Control
Dynamac Corporation

Signature: Finis Cavender
Date: 10/31/85

Jane Harris, Ph.D.
EPA Reviewer and Section Head

Signature: Jane E Harris
Date: 11/1/85

7. SUMMARY:

A group of ten male and ten female randomly selected CrL: CD(BR)SD rats (Charles River, Kingston, Stone Ridge, NY) weighing between 189 and 228 g were exposed for a single 4-hour period to RH-53,866 40WP in an inhalation chamber under dynamic conditions at a measured mean aerosol concentration of 5.0 mg/L. Table 1, from pg. 7 of the CBI, summarizes the inhalation chamber conditions. The animals were observed for signs of intoxication at least two times per hour during the exposure period and twice daily for 2 weeks post-exposure, except on weekends when animals were checked for mortality only. Body weights were recorded prior to exposure and on days 1, 3, 5, 7, 11, and 14 of the post-exposure period. All animals were sacrificed and necropsied at the end of the 14-day observation period. No mortalities occurred during this study. During the 4-hour exposure, the rats exhibited signs of intoxication which included salivation, lacrimation, eye squint, rhinorrhea, bradypnea, and dyspnea. However, the rats were not visible after 2 hours because of the high concentration of dust in the chamber. Immediately after exposure and for the remainder of Day 0, the rats were covered with the test substance, and continued to show signs of toxicity including bradypnea, dyspnea, decreased motor activity, and eye squint. Gasping, ataxia, rhinorrhea, and/or dry corneas were also observed in at least one animal. The majority of signs of intoxication disappeared by the end of Day 2 in both males and females. All animals appeared normal after Day 7 with the exception of corneal opacities in some males and red exudate around the eyes of one female on Day 8. The signs of intoxication observed during and following exposure indicated that the test substance was a slight sensory irritant. Table 2 summarizes the mean body weight data and the body weight changes from Day 0. On Day 1, males and females had mean body weight losses of 10 and 4 percent, respectively. By Day 3, mean body weights of both the males and females were equal to their pre-exposure mean body weights. At the end of the 14-day observation period, males and females had mean body weight gains from Day 0 (starting weights) of 47 and 15 percent, respectively. At necropsy, five males and one female rat had corneal opacities, and two female rats had rough-appearing corneas. These were the only lesions observed that were related to exposure to the test substance.

Based on these data, rats exposed to a 5.0 mg/L dust aerosol concentration of RH-53,866 40WP exhibited signs of slight sensory irritation, corneal opacities, and effects on body weight gains. The LC_{50} for RH-53,866 40WP was estimated to be greater than 5.0 mg/L of air, which corresponds to Toxicity Category III.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

A. The study design was adequate and the data appeared to be valid. A quality assurance statement, dated May 21, 1984, was present.

231

MYCLOBUTANIL 128857

Page_____ is not included in this copy.

Pages 235 through 236 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

9. CLASSIFICATION:

Core Classification: Core guideline.
Toxicity Cateogry: III.
LC_{50} (males and females): Greater than 5.0 mg/L.

EPA: 68-01-6561
TASK: 123
September 11, 1985

DATA EVALUATION RECORD

RH-53,866

Primary Dermal Irritation Study in Rabbits

STUDY IDENTIFICATION: Krzywicki, K. M., and Morrison, R.D. Acute skin irritation, definitive, rabbits. (Unpublished study No. 84R-082A prepared and submitted by Rohm and Haas Co., Spring House, PA; dated July 16. 1984.) Accession No. 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 9-11-85

1. CHEMICAL: RH-53,866 40WP.

2. TEST MATERIAL: RH-53,866 40WP, from lot No. EG-0809-1, TD No. 84-039, is described as a white powder consisting of 39.5 percent active ingredient.

3. STUDY/ACTION TYPE: Primary dermal irritation study in rabbits.

4. STUDY IDENTIFICATION: Krzywicki, K. M., and Morrison, R. D. Acute skin irritation, definitive, rabbits. (Unpublished study No. 84R-082A prepared and submitted by Rohm and Haas Co., Spring House, PA; dated July 16, 1984.) Accession No. 072896.

5. REVIEWED BY:

Peggy Perreault, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Peggy Perreault
Date: 9-10-85

Patricia Turck, M.S.
Independent Reviewer
Dynamac Corporation

Signature: Patricia Turck
Date: 9/10/85

6. APPROVED BY:

Finis Cavender, Ph.D.
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Date: 9/10/85

Jane Harris, Ph.D.
EPA Reviewer and
Section Head

Signature: Jane E Harris
Date: 9/11/85

7. SUMMARY:

A group of six male New Zealand white rabbits (Hazleton Dutchland, Denver, PA) received single dermal doses of 0.5 g of RH-53,866 40WP prepared as a paste in saline (1:2). A patch-test technique was used on intact skin that had been clipped free of hair. After a continuous 4-hour exposure, the patches were removed and the test material was gently wiped off; however, some of the test material remained on the test site. The skin reaction (erythema and edema) was evaluated using a grading scale at 1, 24, and 72 hours, and 7 days after the initial 4-hour exposure. Very slight erythema was noted in five animals 1 hour after exposure, which disappeared in four animals by 72 hours. Desiccation was observed in two animals 7 days after exposure. The 72-hour mean irritation score (MIS) was 0.2. Based on these data, the test substance, RH-53,866 40WP, was slightly irritating to the skin of rabbits; the 72-hour MIS was determined to be between 0 and 2, which corresponds to Toxicity Category IV.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The study design was adequate and the data appeared to be valid; however, a quality assurance statement was not presented. A statement verifying that good laboratory practices were to be followed was included, but it was not signed and dated.

9. CLASSIFICATION:

Core Classification: Core Minimum.

Toxicity Category: IV.

RH-53,866 40WP is a mild dermal irritant.

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

004937

EPA: 68-01-6561
TASK: 123
September 18, 1985

DATA EVALUATION RECORD

RH-53,866

Acute Dermal Toxicity Study in Rabbits

STUDY IDENTIFICATION: Krzywicki, K. M., and Morrison, R. D. Acute dermal LD_{50}, definitive, rabbits. (Unpublished study No. 84R-082A and 84R-082B prepared and submitted by Rohm and Haas Co., Spring House, PA; dated July 16, 1984.) Accession No. 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 9-17-85

1. CHEMICAL: RH-53,866 40WP.

2. TEST MATERIAL: RH-53,866 40WP, from lot No. EG-0809, TD No. 84-039, is described as a white powder containing 39.5 percent active ingredient.

3. STUDY/ACTION TYPE: Acute dermal toxicity study in rabbits.

4. STUDY IDENTIFICATION: Krzywicki, K. M., and Morrison, R. D. Acute dermal LD_{50}, definitive, rabbits. (Unpublished study No. 84R-082A and 84R-082B prepared and submitted by Rohm and Haas Co., Spring House, PA; dated July 16, 1984.) Accession No. 072896.

5. REVIEWED BY:

Peggy Perreault, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Peggy Perreault
Date: 9-17-85

Pat Turck, M.S.
Independent Reviewer
Dynamac Corporation

Signature: Pat Turck
Date: 9/17/85

6. APPROVED BY:

Finis Cavender, Ph.D.
Acute Toxicity
Technical Quality Control
Dynamac Corporation

Signature: Finis Cavender
Date: 9/17/85

Jane Harris, Ph.D.
EPA Reviewer and
Section Head

Signature: Jane E Harris
Date: 9/18/85

7. SUMMARY

A group of six male and six female New Zealand white rabbits (Hazleton Dutchland, Denver, PA), weighing between 2.08 and 2.10 kg, received a continuous 24-hour dermal dose of 5.0 g/kg RH-53,866 40WP, prepared as a paste in saline (1:1.5). The rabbits were observed for 14 days. All male and female rabbits survived through day 14. One male rabbit had scant droppings during day 2 but recovered by day 3. Moderate skin irritation (erythema and edema) was observed on day 1 and disappeared by day 7; no other toxic signs were observed. All animals were sacrificed at the end of the observation period and no gross changes were observed at necropsy. Based on these data, a single dermal application of RH-53,866 40WP is almost nontoxic to male and female rabbits; the dermal LD_{50} is greater than 5.0 g/kg for both male and female rabbits, which corresponds to Toxicity Category III.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The study design was adequate and the data appeared to be valid; however, a quality assurance statement was not presented. A statement that good laboratory practices were to be followed was included, but it was not signed and dated.

9. CLASSIFICATION:

Core Classification: Core Minimum.

Toxicity Category: III.

Dermal LD_{50}: > 5.0 g/kg for both male and female rabbits.

004937

EPA: 68-01-6561
TASK: 123
September 11, 1985

DATA EVALUATION RECORD

RH-53,866

Acute Oral Toxicity Study in Rats

STUDY IDENTIFICATION: Krzywicki, K. M., and Morrison, R. D. Acute oral LD_{50}, definitive, rats (M&F). (Unpublished study Nos. 84R-082A and 84R-082B prepared and submitted by Rohm and Haas Co., Spring House, PA; dated July 16, 1984.) Accession No. 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: [signature]

Date: 9-11-85

RH-53,866
40 W

1. CHEMICAL: RH-53,866 40WP.

2. TEST MATERIAL: The test substance, RH-53,866 40WP, from lot No. EG-0809-1, TD No. 84-039, was a white powder containing 39.5 percent active ingredient.

3. STUDY/ACTION TYPE: Acute oral toxicity study in rats.

4. STUDY IDENTIFICATION: Krzywicki, K. M., and Morrison, R. D. Acute oral LD_{50}. definitive, rats (M&F). (Unpublished study Nos. 84R-082A and 84R-082B prepared and submitted by Rohm and Haas Co., Spring House, PA; dated July 16, 1984.) Accession No. 072896.

5. REVIEWED BY:

Peggy Perreault, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Peggy Perreault
Date: 9-10-85

Patricia Turck, M.S.
Independent Reviewer
Dynamac Corporation

Signature: Patricia Turck
Date: 9/10/85

6. APPROVED BY:

Finis Cavender, Ph.D.
Acute Toxicity
Technical Quality Control
Dynamac Corporation

Signature: Finis Cavender
Date: 9/10/85

Jane Harris, Ph.D.
EPA Reviewer and
Section Head

Signature: Jane E Harris
Date: 9/11/85

7. SUMMARY:

A range-finding study was conducted with groups of 10 male and 10 female CRCD rats (Charles River, Inc.) weighing between 179 and 194 g. The rats were gavaged with RH-53,866 40WP dispersed in distilled water at a constant volume of 20 mL/kg at dose levels of 5.0 and 1.0 g/kg. The rats were observed for 14 days. The results of this study were combined with an additional, definitive study. The additional study was conducted to determine the LD_{50} with groups of 10 male and 10 female rats weighing between 165 and 197 g at dose levels of 5.00 (a combined total of 20 rats/sex at this dose level), 3.34, 2.24, and 1.19 g/kg. A control group of 10 rats/sex was gavaged with 20 mL/kg of distilled water. Rats were fasted overnight prior to dose administration. A total of 15, 10, 9, and 3 male rats gavaged with 5.0, 3.34, 2.24, and 1.19 g/kg, respectively, died by day 4 of the observation period and 17, 8, 8, and 2 female rats at the same dose levels died by day 1. All control rats and rats dosed with 1.00 g/kg of RH-53,866 40WP survived through day 14. Pharmacotoxic signs included passiveness, ataxia, convulsions, prostration, red- and/or tan-stained muzzle, salivation, abdominal breathing, diarrhea, scant droppings, and brown- and/or yellow-stained anogenital area. At necropsy, animals that died during the study had reddened lungs and intestines, stomachs filled with a white viscous or tan solid material, and dark livers; animals sacrificed at the end of the observation period exhibited no gross pathology.

Based on these data, a single oral dose of RH-58,866 40WP is slightly toxic to rats; the oral LD_{50} for male rats was 1.87 (1.26-2.50) g/kg and for female rats was 2.09 (1.56-2.69) g/kg, which corresponds to Toxicity Category III.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The study design was adequate and the data appeared to be valid; however, a quality assurance statement was not presented. A statement that good laboratory practices were to be followed was included, but it was not signed and dated.

9. CLASSIFICATION:

Core Classification: Core Minimum.

Toxicity Category: III.

LD_{50} = 1.87 g/kg (males).

LD_{50} = 2.09 g/kg (females).

00497

EPA: 68-01-6561
TASK: 123
September 11, 1985

DATA EVALUATION RECORD

RH-53,866

Primary Dermal Irritation Study in Rabbits

STUDY IDENTIFICATION: Krzywicki, K. M., and Watts, M. H., Jr. Acute skin irritation, definitive, rabbits. (Unpublished study No. 84R077A submitted and prepared by Rohm and Haas Co., Spring House, PA; dated July 16, 1984) Accession No. 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 9-11-85

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1. CHEMICAL: RH-53,866 2EC.

2. TEST MATERIAL: RH-53,866 2EC (emulsifiable concentrate formulation), material key 892334-7, TD No. 84-027, lot No. EG-0807-1, was described as a liquid containing 28.5 percent RH-53,866 Tech (24.0 percent active ingredient).

3. STUDY/ACTION TYPE: Primary dermal irritation study in rabbits.

4. STUDY IDENTIFICATION: Krzywicki, K. M., and Watts, M. H., Jr. Acute skin irritation, definitive, rabbits. (Unpublished study No. 84R077A submitted and prepared by Rohm and Haas Co., Spring House, PA; dated July 16, 1984.) Accession No. 072896.

5. REVIEWED BY:

Peggy Perreault, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Peggy Perreault
Date: 9-11-85

Sharon M. Ambrose, B.S.
Independent Reviewer
Dynamac Corporation

Signature: Sharon M. Ambrose
Date: 9-11-85

6. APPROVED BY:

Finis Cavender, Ph.D.
Acute Toxicology
Technical Quality Control
Dynamac Corporation

Signature: Finis Cavender
Date: 9/16/85

Jane E. Harris, Ph.D.
EPA Reviewer and
Section Head

Signature: Jane E Harris
Date: 9/11/85

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7. SUMMARY:

A group of six male New Zealand white rabbits (Hazleton Dutchland, Denver, PA) was treated continuously for 4 hours with a single dermal dose of 0.5 mL of RH-53,866 2EC. The test material was applied to the shaved skin of the rabbits; each rabbit was wearing an impervious cuff. The area was then covered with a patch. Application sites were scored (by the Mean Irritation Score) 1, 24, and 72 hours and 7 and 14 days after exposure. One hour after removal of the patches, the rabbits exhibited moderate erythema (mean value = 2.8) and very slight to slight edema (mean value = 1.3). Skin reaction became more pronounced 24 hours after exposure with moderate to severe erythema (mean value = 3.5), eschar formation, and slight to moderate edema (mean value = 2.3). Seventy-two hours after exposure, animals exhibited severe erythema (mean value = 4.0) and slight to moderate edema (mean value = 2.3), resulting in a total 72-hour Mean Irritation Score of 6.3. The animals also exhibited eschar formation, desiccation, and blanching. By day 7, erythema and edema were imperceptible and eschar sloughed off with no new hair growth underneath. On day 14, one animal still exhibited desiccation. Based on these data, the test substance, RH-53,866 2EC, is severely irritating to the skin of rabbits; the 72-hour Mean Irritation Score was greater than 5, corresponding to Toxicity Category II.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The study design was adequate and the data appeared to be valid; however, a Quality Assurance Statement was not presented.

9. CLASSIFICATION:

Core Classification: Core Minimum.

Toxicity Category: II.

Severe Dermal Irritant.

EPA: 68-01-6561
TASK: 123
September 11, 1985

DATA EVALUATION RECORD

RH-53,866

Acute Dermal Toxicity Study in Rabbits

STUDY IDENTIFICATION: Krzywicki, K. M., and Murphy, M. E. Acute dermal LD_{50}, definitive, rabbits. (Unpublished study Nos. 84R-077A and 84R-077B prepared and submitted by Rohm and Haas Co., Spring House, PA; dated July 16, 1984.) Accession No. 072896.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 9-11-85

1. CHEMICAL: RH-53,866 2EC.

2. TEST MATERIAL: RH-53,866 2EC, TD No. 84-027, lot No. EG 0807-1, was described as a brown liquid containing 28.5 percent RH-53,866 Tech (24.0 percent active ingredient).

3. STUDY/ACTION TYPE: Acute dermal toxicity study in rabbits.

4. STUDY IDENTIFICATION: Krzywicki, K. M., and Murphy, M. E. Acute dermal LD_{50}, definitive, rabbits. (Unpublished study Nos. 84R-077A and 84R-077B prepared and submitted by Rohm and Haas Co., Spring House, PA; dated July 16, 1984.) Accession No. 072896.

5. REVIEWED BY:

Peggy Perreault, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Peggy Perreault
Date: 9-1-85

Sharon Ambrose, B.S.
Independent Reviewer
Dynamac Corporation

Signature: Sharon Ambrose
Date: 9-10-85

6. APPROVED BY:

Robert J. Weir, Ph.D.
Acute Toxicity
Technical Quality Control
Dynamac Corporation

Signature: Robert J. Weir
Date: 9-11-85

Jane Harris, Ph.D.
EPA Reviewer and
Section Head

Signature: Jane E. Harris
Date: 9/12/85

7. SUMMARY:

Four groups of six male and six female New Zealand white rabbits (Hazleton Dutchland, Denver, PA), weighing between 2.03 and 2.17 kg, received single dermal applications of 5.00, 3.68, 2.71, or 2.00 g/kg of RH-53,866 2EC, as received, on intact, clipped skin for 24 continuous hours. The application site was covered with an impervious cuff. After 24 hours of exposure, the cuffs were removed and the application site was wiped and examined for irritation. The rabbits were observed for 14 days post-exposure. One male rabbit treated with 5.00 g/kg died on day 6 of the observation period, and three female rabbits treated with 5.00 g/kg died by day 4. All male and female rabbits treated with 3.68, 2.71, and 2.00 g/kg survived through day 14. Signs of toxicity consisted of passiveness and scant droppings in all dosage groups. Other occasional signs included ataxia, tremors, abdominal breathing, gasping, vocalization, nasal discharge, dark brown urine, and blood on the dropping sheet. At necropsy, the male rabbit that died had an eschar at the application site and a fluid-filled thoracic cavity. The females that died had pale livers and black fluid-filled, distended, and/or ulcerated stomachs. Animals sacrificed at the end of the observation period exhibited eschar and/or scar formation at the application site. Based on these data, the acute dermal LD_{50} of RH-53,866 2EC in male rabbits is greater than 5.0 g/kg and in female rabbits is approximately 5.0 g/kg, which corresponds to Toxicity Category IV.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The study design was adequate and the data appeared to be valid. A quality assurance statement was not presented. A statement that good laboratory practices were to be followed was included; however, it was not signed and dated.

9. CLASSIFICATION:

Core Classification: Core Minimum.

Toxicity Category: IV

Acute Dermal LD_{50}: Male rabbits-- >5 g/kg.
: Female rabbits-- ~5 g/kg.